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# The Journal of the
# International College of Surgeons

SIXTH INTERNATIONAL ASSEMBLY
of the
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at the Invitation of the Italian Gov
ROME, ITALY
May 16-24, 1948

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Official Journal of the International College of Surgeons Published Bimonthly
PUBLICATION OFFICE 372 BROADWAY ALBANY 7, N Y
Editorial and General Offices 850 West Irving Park Road Chicago 13 Ill
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Official Journal of the International College of Surgeons Published Bimonthly
PUBLICATION OFFICE, 372 BROADWAY ALBANY 7, N Y
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# The Journal of the International College of Surgeons

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# Original Articles

## Impending Death Under Anesthesia*

HAMILTON BAILEY, F.R.C.S. (Eng.), F.A.C.S., F.I.C.S.† (Hon.)
LONDON, ENGLAND

THE expectation of death under an anesthetic is [illegible] which every surgeon is bound to encounter, as I per[illegible] when consulted on a very large number of cases from five teaching hospitals on th[illegible] continents. Most emergency surgeons are removed from the facilities of a teaching hospital, their patients are often critically ill and must be prepared for operation hastily. For these reasons an emergency surgeon comes face to face with impending death under anesthesia relatively often.

My introduction to this important subject was when, as a student, I was called upon to help in an unavailing attempt to revive a girl who had collapsed under nitrous oxide anesthesia. Since that time I have often reflected how profitable it would be to have organized drill to prepare us for these cases. Unfortunately, with an operating room staff changing constantly, this is seldom practical.

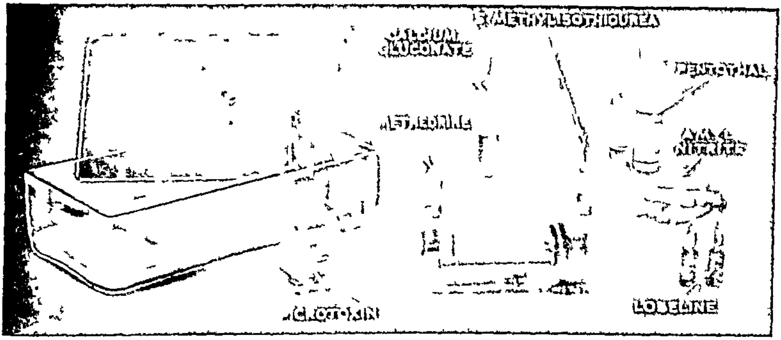

Fig. 1. Anesthetic Emergency Outfit. (a) Methedrine (general pressor substance). (b) Amyl nitrite (cardiac arrest or cardiac depression). (c) Alphalobeline (respiratory center stimulant). (d) Calcium gluconate (ether convulsions). (e) Pentothal (anesthetic convulsions). (f) Picrotoxin (antidote to pentothal). (g) S-methylisothiourea (collapse under spinal anesthesia).

To take the trouble to procure a distinctive jar and place within it certain resuscitative agents, to seal it with adhesive tape, to label it "Anesthetic Emergency Outfit," and to know where it is kept in the operating room is not asking much. It is worth while overcoming the inertia that alone prevents these agents (Fig. 1) from being at hand when they are needed so desperately. To procrastinate will assuredly lead to regret.

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* Read at Eleventh Annual Assembly, U. S. Chapter, International College of Surgeons, Detroit, Michigan, October, 1946.

† Surgeon, Royal Northern Hospital, London.

Those who stop breathing and give rise to anxiety while under general anesthesia can be segregated into two fundamental categories:

(1) Blue asphyxia (primarily respiratory)
(2) White asphyxia (primarily cardiac)

It is to the far more serious condition of white asphyxia that special attention will be directed.

## WHITE ASPHYXIA

*Technic of cardiac massage:* With a sweep of the knife the upper abdomen is entered in the middle line immediately beneath the xiphoid sternum. The incision must be long enough to admit the hand. When a counterpressure is exerted by the base of the left palm on the lower left costal margin, effective intermittent pressure can be exerted on the ventricles by the pulp of the right hand beneath the diaphragm (Fig. 2). For the first half minute these movements should be quick and forcible. After half a minute (so as not to overtire the hand) the movements should become slower. Sometimes one cardiac contraction takes place but the heart does not continue to beat. In nearly all such cases it requires only a little further perseverance to experience the incredible satisfaction

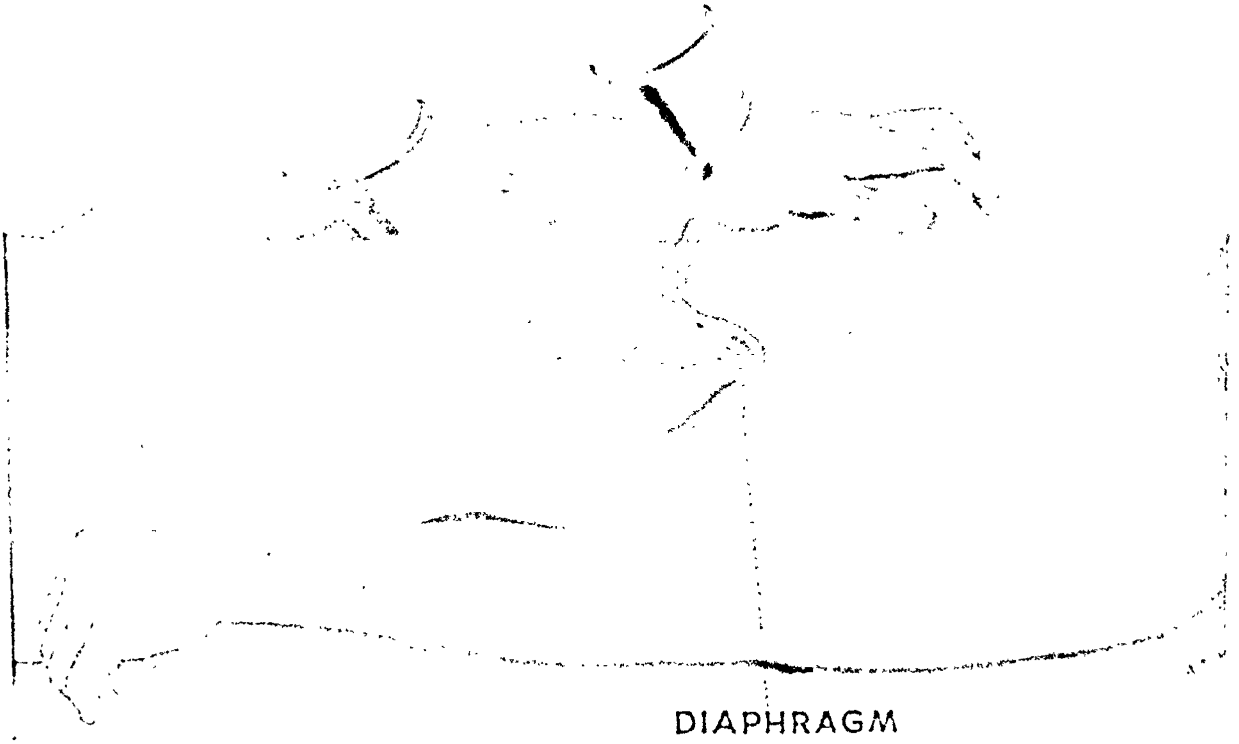

Fig. 2. Standard method of performing cardiac massage.

of feeling the heart spring into automatic activity. The beats strengthen, they strengthen so quickly that by the time the surgeon has given a sigh of relief the contractions become so strong that the heart knocks against the tired hand beneath the diaphragm. Whatever may be the travails that accrue from the choice of the practice of surgery as a profession, thrice certain is it that in no walk of life can there be a greater reward of assiduous labor than that of seeing before one's eyes life itself returning.

In each of my cases subdiaphragmatic massage, just described, was used.

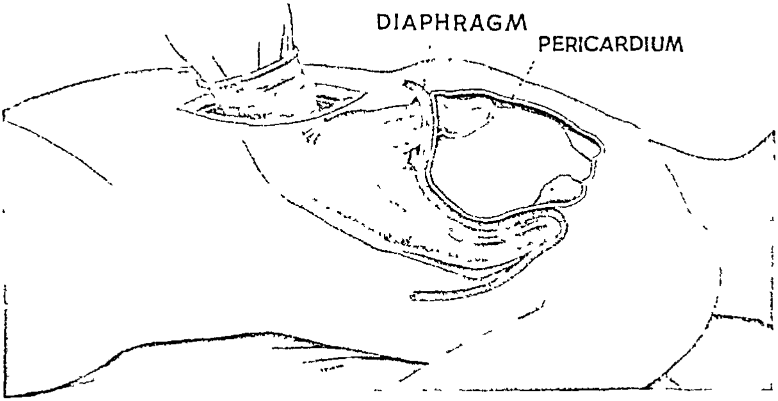

Fig. 3. Nicholson's method of performing cardiac massage

When the patient has a deformed thorax or a rigid costal margin (usually such patients are elderly) and as a consequence the operator is not satisfied that he is compressing the ventricles effectively, or when no response to the standard method has been obtained after a minute or a minute and a half, Nicholson's[6] method (Fig. 3) should be adopted. Some surgeons employ Nicholson's method from the start, and their argument for so doing is well founded.

*Nicholson's Method:* A button-hole incision is made immediately behind the base of the xiphisternum between the attachment of the two sides of the diaphragm. The thumb of the right hand is thrust through the opening. The heart (within its pericardium) can then be compressed between the thumb above and the fingers beneath the diaphragm.

* * *

For more than 20 years I have endeavored to clarify what is the best procedure in cases of cardiac arrest. In former editions of my "Emergency Surgery" I set out a table: a timekeeper, artificial respiration, hot precordial packs, and intracardiac injection of adrenalin were scheduled in an orderly plan of action. In that plan (now obsolete) cardiac massage might be described as the last card.

From time to time I have questioned myself whether these instructions, which conformed to orthodox surgical teaching, were in the best interests of the patient. An analysis of published cases showed that cardiac massage had been relatively more successful when the catastrophe necessitating it had occurred during the course of an abdominal operation. It was reasonable to assume that, the abdomen being open, massage was resorted to sooner than would otherwise have been the case. In 1941 I recorded that I had performed cardiac massage about 40 times. In 13 cases the heart started beating in earnest; but too often, after giving hope for hours or days, disappointment was the outcome—only 5 patients survived. The following case caused me to revise the plan and to advocate earlier cardiac massage.

I had completed the operation of epididymectomy for tuberculosis on a boy aged 12. No anxiety had been experienced. I happened to look through the window of the changing-room, and saw the anesthetist performing artificial respiration. The child had white asphyxia, and we immediately put in operation the routine of having a timekeeper call out the passing minutes. At three-and-a-half minutes I injected adrenaline into the heart. At four-and-a-half minutes, after hastily scrubbing up, I opened the upper abdomen and performed cardiac massage. A full minute lapsed without any response. There was then one beat, a short interval, then three or four beats, and he commenced to breathe. By the time the abdomen had been closed with through-and-through sutures some color had returned to the patient's blanched face, the pulse was full, but very rapid, and oxygen was being administered. Next day the patient said he felt well.

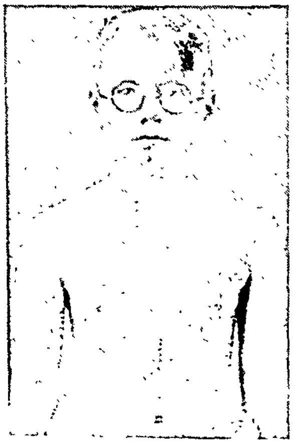

Fig. 4. Case described, six months after successful cardiac massage. Vision unimpaired.

On the third day the boy complained of dimness and double vision. On the fourth day he could distinguish only between light and darkness. There were other evidences *of serious disturbance of the central nervous* system, such as incoördination and some paresis. Obviously the vital centers had been left too long without a blood supply, and during the period of blindness I wondered whether it would not have been better if the cardiac massage had failed. It was during this period that I became determined to advocate earlier cardiac massage. By what I believe to be an unprecedented stroke of good fortune, the patient's vision gradually returned and all other untoward signs disappeared (Fig. 4). This patient was young, and it is reasonable to suppose that the unexpected restitution of function was attributable to his youth, for Swift Joly's[5] patient, a man of 55, resuscitated under similar circumstances, never fully recovered.

I want to make it perfectly clear that I have now abandoned expending time in performing artificial respiration and injecting adrenaline into the ventricle. It seems obvious that, if the heart has stopped, the sooner it is massaged the better. As a consequence of following this simple dictum I am able to record three consecutive successful cases of cardiac massage, an experience vastly different from that when cardiac massage was longer delayed.

Further, it appears that the early anxieties and later complications that follow temporary cardiac cessation are proportionate to the length of time the organ is functionless—an observation in keeping with rudimentary physiological principles and common sense. It may even be said that, with prophylactic chemotherapy now available, if a patient is in reasonably good condition before being anesthetized and the time the heart is allowed to stand still is less than 1½ minutes, that patient will probably suffer neither harm nor even any inconvenience beyond that occasioned by an upper abdominal incision

### CURRENT PLAN OF ACTION

The anesthetist reports that he cannot feel the pulse Is the heart beating? When the surgeon has access to a large artery such as the abdominal aorta or the common iliac artery, he can himself confirm or deny that the heart has ceased to beat When such an artery is not available, it is the anesthetist who must settle this point by the means he thinks best, in my view the carotid pulse is the most reliable indicator Three quarters of a minute is available for obtaining this information During this period the anesthetist verifies that the patient's airway is not obstructed This being done, he breaks a capsule of amyl nitrite under the patient's nostrils According to Primrose, the exhibition of amyl nitrite is the means of distinguishing between cardiac depression and cardiac arrest While these 45 seconds are fleeting, the surgeon is not idle, he and the theater sister or surgical nurse are sterilizing the precordial and epigastric areas, and arranging towels suitably

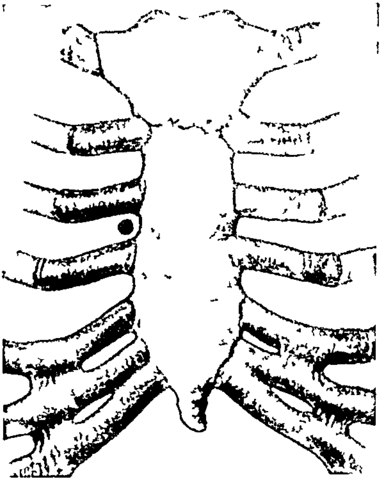

Fig 5 Illustrating point at which auricle should be pricked

*Pricking the auricle* As soon as the precordial area has been sterilized, the right auricle should be pricked with a needle The needle is thrust into the third right interspace at the upper rim of the fourth rib, close to the sternal border (Fig 5), and is directed upwards (Hyman[8]) This can be accomplished in the twinkling of an eye

*Cardiac massage* Unless the response to the above stimulus is obvious and immediate, cardiac massage by the technic described above must be undertaken forthwith

*Posture* The head should be lowered by tilting the operating table "The cortex can be kept from death by the merest trickle of blood" (Sir Leonard Hill) The anesthetist can attend to this while cardiac massage is in progress

*Artificial respiration* Manifestly, artificial respiration is futile if the heart is not beating, but no harm is done in continuing automatic respiration with an anesthetic machine if the anesthetist has the facilities for doing this Indeed such a measure is highly desirable, for, should the heart's action be re-established, not a second is lost in oxygenating the erstwhile stagnant blood in the lungs

In some instances, almost as soon as the heart starts beating the patient breathes spontaneously. In others artificial respiration must be carried out sometimes for a long period.

*Repairing the incision.* It is inadvisable to close the midline incision until it is certain that the patient is breathing without artificial aid. If the surgeon has time on his hands, he can insert through- and through sutures without tying them (Fig. 6).

Sulfanilamide powder is poured into the wound, which is closed rapidly. It is sufficient to close the upper midline incision with strong, interrupted through-and-through sutures traversing all layers. If the button-hole incision in he diaphragm has been made, the suture of this opening is unnecessary.

*Immediate after-care.* As soon as convenient, the blood-pressure is taken; if it is under 100 mm. Hg, plasma infusion is given and a pressor drug administered.

*More remote after-care.* An important consideration in the after-care of the patient is that his lungs should be kept well ventilated by the intermittent administration of $CO_2$. Whenever possible, penicillin therapy should be started within the first few hours. These measures are designed to prevent pulmonary complications that are so likely to develop after trauma of the diaphragm.

## SOME ENCOURAGEMENTS

How long should we persevere with cardiac massage? This is a question that has never been settled, but a lead is given from a case reported by Adams and Hand,[10] where cardiac massage was continued for no less than twenty minutes before a response was obtained. The patient recovered.

Perhaps the most stimulating case is that recorded by Easton,[11] who courageously performed cardiac massage by Nicholson's [6] method in circumstances which allowed only of using an unsterilized scalpel, with his hands unwashed. His patient was a woman, aged 25, whose heart stopped during chloroform anesthesia for childbirth. Several hours after this ordeal the patient had recovered sufficiently for a general anesthetic to be administered while her abdominal

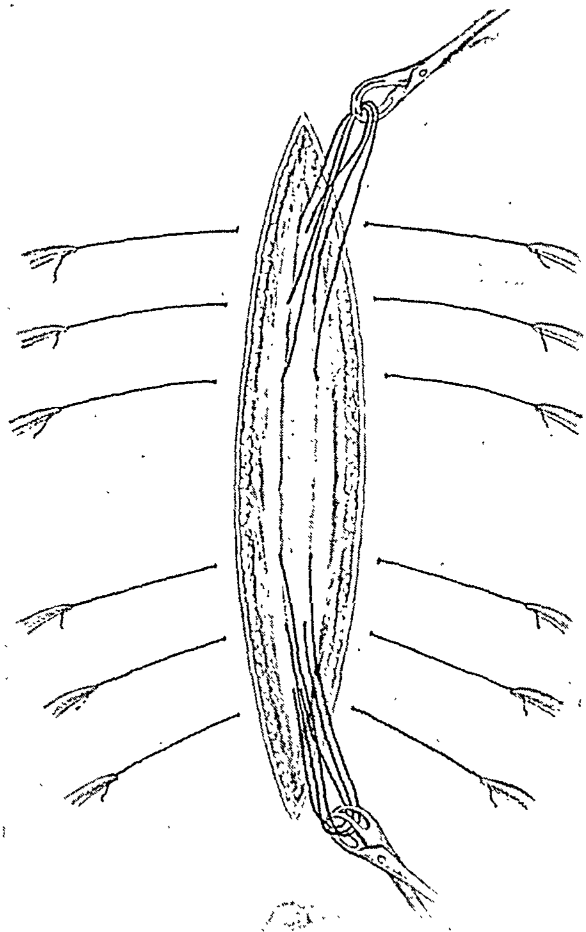

Fig. 6. Through and through sutures having been inserted, they can be gathered together as shown, and later tied in a few seconds.

wall was sutured. She recovered uneventfully.

MacLeod's[12] patient collapsed under spinal anesthesia. Though the heart responded to cardiac massage after two minutes, it was 45 minutes before respirations became automatic. Throughout this period the surgeon and the assistant took turns in carrying out artificial respiration and keeping a hand beneath the diaphragm.

Grimshaw's[13] patient was a woman, aged 61, whose heart stopped during the administration of pentothal. Cardiac massage performed about three minutes later led to complete recovery.

These reports prove that cardiac massage can bring back to life those whose hearts have ceased to beat while under any form of anesthesia.

## BLUE ASPHYXIA

Concerning the possibilities of resuscitation in cases where the heart's action has ceased following blue asphyxia, little information is to be found in literature. I am sure that I have established conclusively that the time limit for successful cardiac massage in blue asphyxia is lengthened tremendously; this is in keeping with what we might expect from a theoretical consideration. In blue asphyxia the cerebral mechanism is not deprived of blood; indeed, it is congested, and because of this, the cerebral cells survive longer than if congestion were not present.

### CARDIAC MASSAGE IN LATE BLUE ASPHYXIA

At 7:30 P.M., I was on my way to visit another patient when an agitated nurse ran out of a room saying, "He is very blue." I entered the room, and a man, aged 28, from whom I had removed the left submaxillary salivary gland for calculus about three-quarters of an hour previously, was lying quite still. His face was suffused and deeply cyanotic. I pulled his head and shoulders to the side of the bed, inserted a mouth gag which was on the table, put my right index finger down to the back of his throat, and attempted to perform artificial respiration by placing the base of my left hand over the xiphisternum. As soon as I had established an airway, I recall that I was surprised to see how effective was this method of getting some air in and out of the lungs. After about a dozen compressions he made one respiratory effort. This, the only sign of life, was not repeated. I had sent the nurse to fetch Sister and anyone else available. At last the nurse returned with the news that Sister was at dinner; she was accompanied by another nurse. I exhorted them to find someone. Possibly a minute later, Dr. Marre, a dermatologist, who had come to visit a patient, appeared. Dr. Marre performed artificial respiration while I swabbed out the pharynx.

After about two minutes we concluded that there were no signs of life, and I asked Dr. Marre to go to the theater and fetch a scalpel. This he did, and returned after what seemed to be a very long time, but afterwards we estimated it was five minutes (the theater was on another floor). During all this time I continued to perform artificial respiration. The scalpel was in a bowl of antiseptic; and, having rinsed my hand, which was lacerated by the patient's teeth, in running water, I dipped my hands in the antiseptic and then poured some on the patient's epigastrium.

Having incised in the middle line, I started cardiac massage from beneath the diaphragm, and, after about 1½ minutes there were, as I have experienced before, one or two beats followed by further cardiac cessation. Continuing the massage for some seconds, the heart sprang into activity, and, shortly afterwards, the patient began to breath naturally. Sulfanilamide powder and the instruments for sewing up the abdomen were sent for (by this time numerous helpers had arrived on the scene), and the abdomen was closed.

By 8:30 P.M., the patient was breathing well, the pulse-rate was about 120 and steady, the pupils were widely dilated and fixed, and the conjunctival reflex was absent. The face was still very suffused, and the purple tongue, which had been torn by my endeavor to draw it forward, continued to bleed; consequently the pharynx had to be swabbed out periodically. By midnight the pupils were smaller, but the conjunctival reflexes were still absent. The patient became restless about 12:30 A.M. and periodic attacks of restlessness occurred during the night. At 4:30 A.M., he showed signs of cerebral irritation and had to be held in bed. One quarter grain of morphine was given. By 5 A.M. the pulse-rate was again down to 120, but at 8 A.M. extreme restlessness returned. Another 1/6th grain of morphine was administered. At 10:30 A.M., there was generalized sweating. The blood pressure was 116 systolic, 78 diastolic. The pupils were pin-point and equal, but there was no reaction to light. The limbs were somewhat spastic; and, what was profoundly disappointing, there was no sign of return of consciousness. However, 13½ hours after the cardiac massage, the patient began to swallow, and, from this time on, hope of a successful issue was entertained. He spoke to a nurse 17 hours after resuscitation.

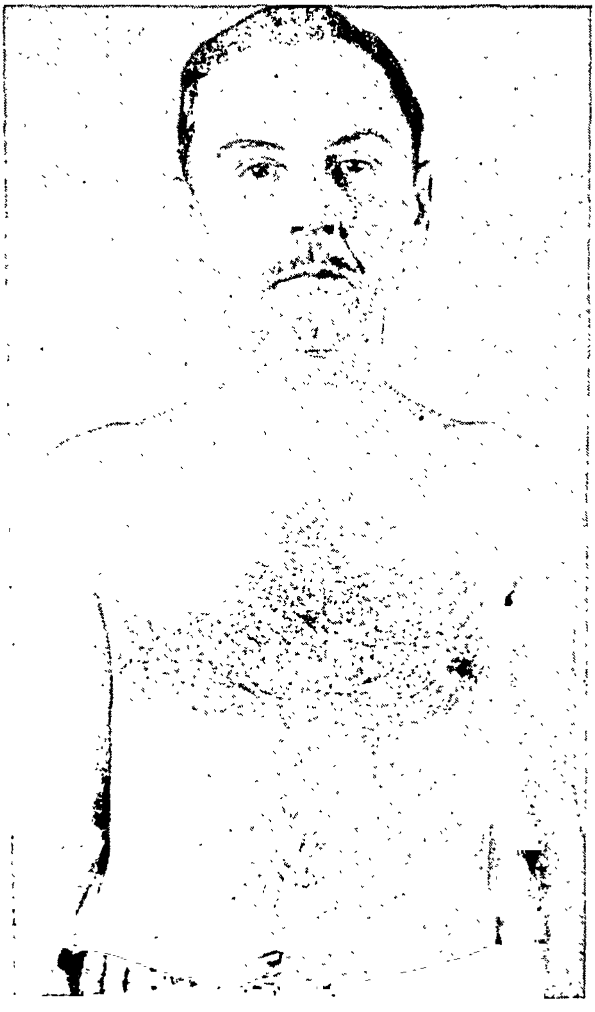
Fig. 7. Case described, two years after remarkable resuscitation. His heart was arrested for at least twelve minutes.

He then continued to improve until the seventh day, when suddenly he experienced a very severe pain in the left side of the abdomen. This was correctly diagnosed (after difficulty) as diaphragmatic pleurisy. A left pleural effusion and later an empyema developed. The patient recovered (Fig. 7).

This case shows that in blue asphyxia the time limit for successful cardiac massage is tremendously lengthened. At the very least, the period of cardiac arrest in this case was 12 minutes; in all probability it was longer.

### SUDDEN VASO-MOTOR COLLAPSE

Referring to those sudden cases of collapse where the pulse becomes almost imperceptible — crises where, to be of value, a pint or more of fluid must be put into circulation in the matter of minutes — it is my opinion that injection of fluid *by means of a syringe or syringes* into the bone-marrow is the method that has yet to be bettered. By means of a sternal-puncture trocar and cannula (Fig. 8) the bone-marrow can

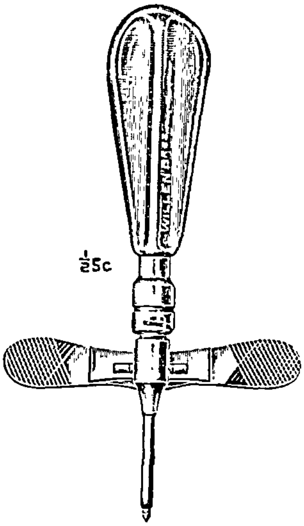

Fig. 8. Sternal trocar and cannula.

be entered with a swiftness and certainty that bedwarfs—nay, bepygmies—cannulization of a radicle of the venous system. I have employed this expedient with unbounded satisfaction in several desperate cases, of which the following is an instructive example.

A very difficult operation had just been concluded—the removal of a fibroneuromyxoma the size of a cricket or tennis ball from the posterior cord of the brachial plexus. In passing attention is called to the marvelous access obtained by Fiolle and Delmas' incision to that deep, dark and dangerous recess behind the clavicle. After removal of this tumour, as the gap in the posterior cord of the brachial plexus was about 6 inches and the ends of the cord had been sutured together, it was considered necessary to put the arm in a shoulder spica plaster. It was while the plaster was being applied that the patient suddenly collapsed. The pulse was imperceptible. After an unfortunate delay in getting ready the apparatus for infusion, it was found that saline solution would not run into the left saphenous vein. The house-surgeon, therefore, cut down on to the right saphenous vein, but the saline solution gravitated at the rate of only about twenty drops a minute. So collapsed was the patient (a woman, aged 33) that it was useless to expect replenishment of the circulating fluid by this route. I therefore ordered a sternal-puncture trocar and cannula to be sterilized rapidly, and forthwith cannulized the *manubrium sterni*. By this time plasma was available, and a pint and a quarter was injected in less than 2 minutes (Fig. 9). After the patient, who appeared moribund, had received a pint of plasma, the anesthetist reported that the pulse was perceptible. It was not until she had received two pints of plasma via the bone-marrow that the cannula tied into the saphenous vein began to function adequately. By this time the patient was out of immediate danger.

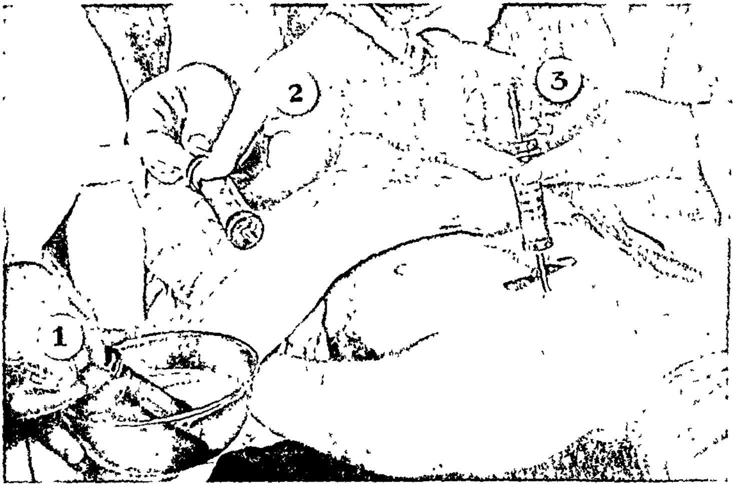

Fig. 9. A pint of plasma or saline solution can be injected by the syringe method into the marrow of the sternum in less than two minutes.

* * *

Impending death under anesthesia is a subject of cardinal importance to every surgeon. Experience has taught me that cardiac arrest can occur in any patient, with any anesthetic agent, and with any anesthetist. For reasons that are obvious, a general emergency surgeon must expect to hear the beating of the wings of the angel of death more often than do surgeons engaged in special branches of our art. Even so, let him who thinketh he standeth take heed lest he fall from unpreparedness. To resolve to prick the auricle within three-quarters of a minute of ascertaining that the heart has ceased to beat; and, in the likely event of this expedient's being ineffective, forthwith to massage the heart, will, I am sure, recall some of those whom Charon has started to ferry across the waters of the Styx.

### SOMMAIRE

L'auteur discute la technique du massage cardiaque dans les cas d'asphyxie blanche ou d'asphyxie bleue, àssociée à arrêt cardiaque, survenant durant le cours de l'anesthésie générale. Il considère cette méthode de ressuscitation comme la première, plutôt que la dernière, ressource. Se basant sur son expérience personnelle, il est d'avis que si on a recours au massage cardiaque dans le délai d'une minute ou d'une minute et demie après que le coeur a cessé de battre, les chances de survie son bien meilleures que si on retarde plus longtemps. It rapporte trois cas consécutifs dans lesquels on suivit ce dictum avec succès. L'auricule est ponctionné trois-quarts de minute après l'arrêt cardiaque. Si cela est sans effet, on aura immédiatement recours au massage cardiaque. Dans les cas d'asphyxie bleue, on a un peu plus de temps car dans ces cas le mécanisme cérébral n'est pas privé de sang. Dans un cas de cette nature, la periode d'arrêt cardiaque était au moins de douze minutes; néanmoins, il y eut réussite.

L'auteur discute aussi l'affaissement vaso-moteur soudain. Il recommande pour ces cas l'injection de fluide dans la moelle osseuse plutôt que par canalisation des veines. Son expérience personnelle avec l'emploi de la ponction du sternum par trocart et canule fui a donné des résultats très satisfaisants.

### RESUMEN

El autor trata de la técnica del masaje cardiaco en los casos de asfixia lívida y pálida, con la concurrencia de interrupción cardíaca que suele ocurrir durante una anestesia general. Cree que este prodecimiento de resurreción debe ser un recurso inmediato, y no postrero. Por propia experiencia llega a la conclusión que el masaje cardíaco debe comenzarse dentro del minuto y medio que sigue a la cesación de latidos en el corazón. En este caso, las oportunidades de revivir la acción del corazón son mucho mejores. El autor registra tres casos consecutivos y con éxito en los cuales siguió el procedimiento mencionado. El aurículo es punzado dentro de los primeros 45 segundos después de la inmovilidad cardíaca, y si se prueba su inefectividad, se comienza el masaje cardíaco inmediatamente.

El tiempo necesario en los casos de asfixia lívida es mayor aún, puea en tales casos el mecanismo cerebral no ha sido desprovisto de sangre. En uno de los casos de que se informa, el período de cesación cardíca fué de 12 minutos y probablemente mayor aún. El resultado fué completamente feliz.

El Dr. Bailey también explica el colapso repentino del vasomotor, recomienda la inyección de flúido por medio

*(Continued on page 27)*

# The Surgical Concept in Hyperthyroidism*

*CHAS GORDON HEYD M D DMSc FACS FICS (Hon)*†
NEW YORK N Y

THE rapid advance of biochemistry has contributed greatly to our knowledge of thyroid disease. The surgical mortality in operations for thyroid disability is surprisingly low. It is conceded that with the exception of cancer of the thyroid, operations for goiter imply no grave operative risk. The low mortality of thyroid surgery is somewhat surprising as the anatomical field is one in which surgical intervention is usually hazardous and fraught with danger. The standardization of technical procedures, the speedy control of hemorrhage, and the low incidence of pulmonary complications have contributed largely to the excellent immediate surgical results.

When we consider postoperative morbidity there is much to be desired in regard to better and more assured good health. Persistent hyperthyroidism, recurrent hyperthyroidism, regrowth of nodular or adenomatous goiters, and disabilities of the voice and breathing mechanism of the larynx are all too frequent and may be the cause of continued ill health or permanent invalidism.

Chemical studies have contributed greatly to our understanding of the fundamental problems of thyroid dysfunction — hyperthyroidism and hypothyroidism. Malignancy of the thyroid is found in almost uniform percentage in all goiter clinics and is basically associated with adenomatous or nodular goiters.

The fact that hyperthyroidism is not infrequently associated with diabetes and tuberculosis has introduced new problems. Chemical studies on the glycolysis of the liver in thyrotoxin stimulation have contributed knowledge on thyroid crises and liver exhaustion. The high percentage of hypoproteinemia in hyperthyroidism has resulted in a more adequate intravenous therapy for this *condition*.

More recently the introduction of thiouracil has opened a new chapter in therapeusis. It may be said that the field of the goiter surgeon has been enlarged by this new knowledge. Let us remember that thiouracil is the first of a group of chemical compounds that may have a decided place in the non operative treatment of hyperthyroidism; we should also recall that the first sulfonamide was extremely dangerous, and that later, with the advance of chemistry and clinical experience, there were evolved the splendid "sulfa" compounds that we use today.

Thiouracil is a very dangerous drug and now that it is on the market it will *be the cause of a number of deaths*— twenty-seven deaths have already been reported following the use of this drug.[1] Its chief danger is the production of an agranulocytosis, which can and undoubt-

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* Read at the Eleventh National Assembly, United States Chapter, International College of Surgeons, Detroit, Michigan, October 22 1946

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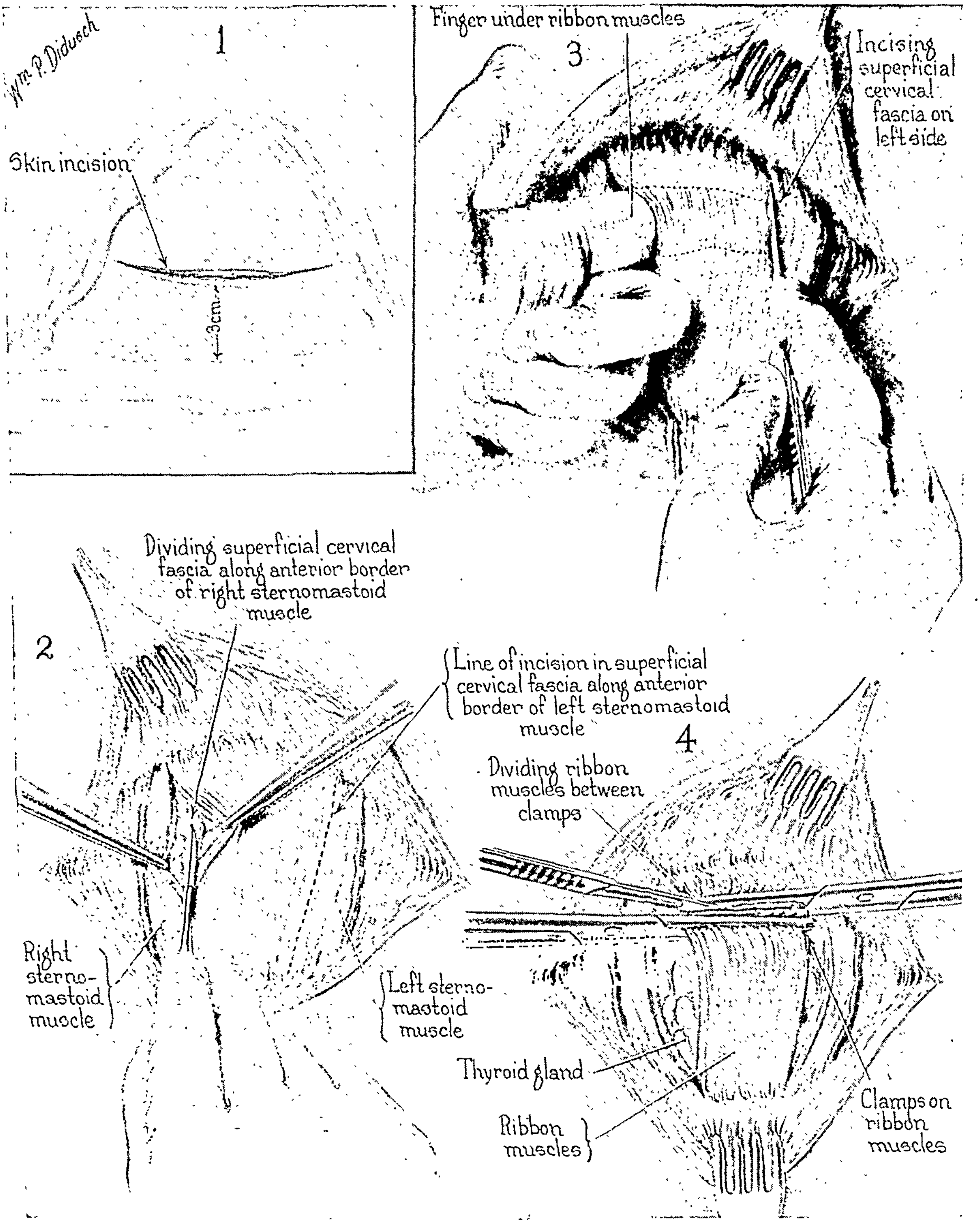
Wm. P. Didusch
1
Skin incision
3cm
3
Finger under ribbon muscles
Incising superficial cervical fascia on left side
2
Dividing superficial cervical fascia along anterior border of right sternomastoid muscle
Line of incision in superficial cervical fascia along anterior border of left sternomastoid muscle
4
Dividing ribbon muscles between clamps
Right sternomastoid muscle
Left sternomastoid muscle
Thyroid gland
Ribbon muscles
Clamps on ribbon muscles

PLATE 1

the time of arrival in the operating room. The sleep is not deep, for the patient may be aroused by being spoken to or by changing his position on the operating table. Ordinarily the patient has no recollection of the trip to the operating room nor of the operation. By this somnolence the preoperative tachycardia produced by [illegible] ion and the trip to the operating room, etc., is avoided. After the patient is placed in position on the operating table, ethylene gas anesthesia is induced.

The usual procedure for exposing the thyroid gland is to incise the cervical fascia in the median line and then to divide the ribbon muscles on either side by applying two crushing clamps at approximately the upper third. When this procedure is completed, it is possible by traction upward and downward of the divided muscle groups, together with lateral traction, to obtain a fairly good but incomplete exposure of either side. However, on many occasions this procedure does not give a good approach to the operative field, and if bleeding occurs it may be extremely difficult to locate or to control without injury to important structures.

In the course of our experience we began to simplify this bilateral procedure by dividing both lateral groups of ribbon muscles—right and left—between two clamps, thus giving a larger and more ample exposure and leaving the operative field less encumbered, with two clamps instead of four. In addition, the goiter and thyroid gland may be seen in their entirety.[5, 6]

The skin incision is placed at least three centimeters above the sternal notch (Plate I, Fig. 1.) It is our custom, before beginning the skin incision to "saw" rapidly with a thread across the greatest anterior protrusion of the thyroid and to make the skin incision either above, through, or below the "red line" made by the thread, depending on the general shape, size, and asymmetry of the goiter. The incision is carried down to but not through the platysma, and the upper and lower flaps are developed. The lower flap is first undermined to a depth of about one to two centimeters, the upper flap, however, is dissected upward for a considerable distance at least above the thyroid prominence. Hemostasis is *completed* before any further operative procedure is carried out. The superficial cervical fascia is divided along the anterior *border of the right* sternomastoid muscle for a distance of seven to ten centimeters (Plate I, Fig. 2.) A Parker retractor is inserted and by lateral traction the right sternomastoid muscle is displaced laterally, exposing the pretracheal fascia which extends from the carotid sheath to the midline. This pretracheal fascia is incised in the same longitudinal direction. The right lateral border of the pretracheal muscles is elevated and the index finger is insinuated below the three ribbon muscles and carried across the midline to the anterior *border of the left* sternomastoid muscle where a similar incision is made through the pretracheal fascia as on the right side (Plate I, Fig. 3.) The muscles are raised off the isthmus of the thyroid and two Kocher clamps are applied as follows: the first assistant, standing on the patient's left side and opposite the operator, inserts the upper Kocher clamp from the patient's left to right side and the operator inserts the lower Kocher clamp from the patient's right to left side, the handles of the Kocher clamps are thus placed one on each side of the patient and the two clamps are applied at approximately the junction of the upper and middle third of the muscle group. The muscles are then divided and with a hook retractor placed under each clamp, traction is made upward and downward, and the entire thyroid area is fully exposed (Plate I, Fig. 4.)

By these procedures a complete exposure is made of the entire thyroid

# The Reconditioning of Spinal Cord Injuries*

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AND
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THE desire and necessity for reconditioning the casualties of the war just ended have created a unique attitude toward their disabilities; and instead of the patients who cannot speak, cannot hear, cannot see, cannot walk being considered as total losses to themselves and their community, such disabling results of combat present a distinct challenge. In investigating all the attempts to overcome such handicaps in previous years, a definite program was planned for the service personnel and carried out by the Medical Departments of the Army, Navy and Air Corps. The information from the Rehabilitation Centers of Great Britain and such civilian projects as that being carried out by The Institute for the Crippled and Disabled was of value in planning and carrying out the reconditioning of spinal cord injuries. It was found that the best results were obtained by coordinating the efforts of the different medical specialties, in determining what was needed in the way of surgical repair requiring the skill of the orthopedist, general surgeon, plastic surgeon, neurologic surgeon, and in the re-education and training requiring the teaching of the physical trainer, the psychologist, the psychiatrist, the speech teacher and the physical therapist.

Brigadier General F. W. Rankin has stated that the results in the care and retraining of patients suffering from spinal cord injuries will remain one of the most important contributions in the field of rehabilitation of casualties from this or any other war. Donald Munro of Boston, who has been one of the great contributors to our knowledge of this problem relating to civilian casualties, stated in a recent article: "If he has been properly treated, every patient with a spinal cord or cauda equina injury who is intelligent and co-operative and has the use of the shoulder, arm and hand muscles can be made ambulatory; can have such control of the bladder and bowels as to sleep through the night without either getting up or wetting himself; can carry out ordinary activities throughout the day without soiling himself with feces or having to evacuate his bladder oftener than once every three hours; can lead a normal social life and, within the limits of his intellectual capacity, can earn a satisfactory living. Such patients can start walking with healed pressure sores and bedsores and, except for a small group, without the need for an inlying catheter or any substitute therefor; they have to use crutches and to wear, when ambulatory, caliper walking splints which, however, permit them to stand or sit. These are the only restrictions to their living the same social life and having the same earning capacity that they had before becoming paralyzed.

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* Presented before the International College of Surgeons, Washington, D. C., December 7, 1945 (Tenth National Assembly).

† The opinions or assertions contained herein are the private ones of the writers, and are not to be construed as official or as reflecting the views of the Navy Department or the Naval Service at large.

One of the important lessons learned from handling the countless casualties was that rehabilitation or reconditioning really started with the first treatment after being wounded, and this was particularly true with the spinal cord injuries. The moment a patient sustains a broken neck or back or any spinal cord injury resulting in paralysis of the lower extremities several constitutional factors complicate any therapeutic effort. Disabling bed and pressure sores will develop if care is not exercised. Urinary infection, dribbling or retained urine due to a paralyzed bladder will cause wet bed clothes. A paralyzed anal sphincter will cause soiling of the bed by feces. Anemia, hypoproteinemia and cachexia may result from inadequate diet, disturbed emotional reaction and possibly "spinal shock" for the first few months after injury.

The immediate care of the patient with spinal cord injuries, of course, comprises transportation to the nearest hospital. Any patient with suspected spinal cord lesion should be carefully rolled onto a stretcher, attempting to prevent any movements which might increase the damage to the spinal cord. Roentgenographic examination of the vertebral column is necessary to determine the bony damage or displacement. However, before any extensive examination is carried out, the associated shock must be overcome. This is done by application of heat and the administration of blood and stimulants. All too frequently a patient is taken to the x-ray examining room before determining the extent of the shock and, as in all emergency cases, the immediate care of the patient is more important than the diagnosis. As soon as the patient has recovered from shock a complete examination is carried out. This comprises a careful neurological examination to determine the extent of the motor and sensory dysfunction. If there are evidences of spinal cord compression in the roentgenograms and a complete transverse lesion of the cord with an absence of motion and sensation of the lower limbs, then a spinal puncture with manometric studies should be performed. A subarachnoid block shows further compression of the cord and indicates an operation. There are some exceptions to this however. Fracture dislocations in the cervical region should be reduced by means of extension with a head harness or traction tongs, and some authorities attempt to reduce fractures in the thoracic and lumbar regions by hyperextension and the application of casts.

Operation upon the spinal cord when the fracture has occurred in the cervical region should be delayed until attempts at extension have been tried. Quite frequently complete reduction can be accomplished and recovery from paralysis be noted within a few days to a week. Should no return of function occur following reduction, then the supposition is that the spinal cord has been irreparably damaged. Surgery is delayed in cervical spinal cord injuries until traction has been attempted, when it may be used to relieve the pressure.

Injuries to the thoracic spinal cord are not so common and usually there is sufficient dislocation of the vertebrae to account for the transverse myelitis. Here again the criteria for operation consist of the amount of dislocation, the results of attempted reduction and the evidence of existing pressure upon the spinal cord by means of the mano in morale which the patients received; with the more intelligent of their patients, the thought of being tied to a catheter the rest of their lives was worse than being paralyzed.

The care of the bowel is of equal importance, as there is a definte paralysis of the sphincter resulting in either incontinence or a tendency to form fecal impactions. Until the bowel makes the adjustment, a diet high in caloric and vitamin content and low in residue should be given. This should be continued at least for a few weeks, while enemas should be given at definite intervals, in an attempt to train the bowel to regular evacuations and thus eliminate involuntary defecation.

There is an interval after every spinal cord injury when a period of observation is necessary to evaluate the amount of permanent damage. In working with casualties it was found that exercises in bed improved their physical condition. Every patient with a spinal cord injury must be convinced that regardless of the extent of his injury he will be able to walk, to move about in a wheel chair and to become independent of a bad existence. A definite lift in his morale is accomplished if this attitude towards his disability is maintained by every one who treats him. It was noticeable in the wards of the service-connected hospitals that these cases were brought in with depressed spirits, listless and uncooperative in eating or in entering into the ward activities. After a few days of observing the others walking down the ward on crutches, wheeling to the bathroom, putting on and taking off the braces, a definite interest occurred and soon they were asking about the physical program which would allow them to do as the others did. The example of others was of more use in the program than all the talking, urging and persuading of the doctors, nurses, social workers or others engaged in their care.

Once their interest had been aroused, they exercised, developed an appetite and co-operated in the care of their bladder and bowels. Munro has shown that pressure sores and bed sores are likely to occur in the cases in which a balanced diet has not been maintained due to a tendency in all these cases to develop an hypoproteinemia; and if the protein balance is maintained, the skin is not so inclined to break down. He emphasizes the turning of the patient in bed and the administration of proteins in preventing these disabling open lesions on the back and hips.

Occasionally these pressure sores occur in spite of all the preventive precautions and remain very resistant to treatment. Plastic operations have been successful with the use of the sulfones and penicillin. When the lesion is small, the ulcerated area can be excised and the skin edges undercut and approximated with tension sutures, as mentioned by White, Hudson, and Kennard. Skin flaps from the adjacent areas can be swung over the area, or split thickness grafts can be applied after the granulating area has been prepared and has become healthy and sterile. These procedures assist in the early healing of these lesions and speed the program of getting the patient into a wheel chair and started on his walking project.

In the meantime the physical therapist can be busy, attempting to prevent stiffening of the joints and maintaining flexibility of the muscles and tendons.

Orthopedic reconstruction operations are rarely necessary in the treatment of traumatic paraplegia. In some instances the spine has been so weakened by the injury or by the removal of bone at the time of neurological exploration that spine fusion is indicated. In carefully selected cases spine fusion will strengthen the back so as to allow the patient to discard back braces and leather jackets and to sit and stand without additional support for the back.

Occasionally a neglected case of paraplegia will develop flexion contractures of the hips, knees and ankles which need to be corrected before the patient can be fitted to braces and be made ambulatory. As a rule, however, those patients with complete paralysis of both legs require no orthopedic operation. Patients with partial paraplegia and only partial loss of motor power in the legs may often be benefited by suitable reconstruction operations. These are usually in the nature of bony stabilizations or tendon transplantations or a combination of both. By carefully planned surgery it is often possible to improve function greatly in the extremity, and in some instances the operations will allow the patient to discard his brace.

In flaccid paralysis there is little difficulty in keeping the muscles and tendons flexible and the joints free from fixation, but in the cases where a spastic paralysis is present, and especially where there are mass reflexes with legs drawn and maintained in the position of flexion and adduction, the problem is more difficult. Again Munro, who has reported a number of successful attempts in treating this type of complication, and the Army and Navy hospitals, where similar procedures have been carried out, present what seems an adequate solution to the problem. As soon as it is established that no other type of conservative treatment will overcome the flexion deformity, a laminectomy is done and the motor components of the spinal nerves on both sides from the tenth dorsal to the second sacral are divided intradurally. This immediately relieves the spasticity and produces a flaccid paralysis, and also allows the application of caliper splints in the training for walking. These caliper splints have lock knee joints which may be unlocked to allow knee flexion when the patient is sitting. The majority of paraplegic patients with complete paralysis of the legs require *long (foot to groin) caliper braces*.

Before the splints are applied, however, the patient should be trained to shift his body about on the bed and then into the wheel chair, for in so doing he learns to balance himself and then can learn to apply the splints and swing himself over the side of the bed to stand upright. This is a difficult lesson for the patient to learn, but it is very important. Balancing on crutches is a difficult feat and is facilitated by a definite program to develop the muscles in the upper extremities which are to bear the brunt of supporting the body on crutches. Much patience is needed by the physical therapist or physical trainer in carrying out the final step in this reconditioning program, for the actual getting about on crutches is the objective of the program. This will take a great deal of time and will need a great deal of encouragement, not only from the doctors, but also from the other patients who have gone through it or are going through the same stages. This is where a grim determination on the part of the patient is of tremendous help and is necessary if the objective is to be reached.

From the standpoint of physical therapy, patients who are to be reconditioned are studied to determine the

moelle épinière. Afin de donner le traitement nécessaire à ces malades, on doit avoir recours à la coopération des spécialties suivantes: orthopédie, chirurgie générale et plastique, psychologie, psychiatrie, thérapie physique et specialiste de parole. Il est de grande importance de soigner immédiatement les conditions difficiles qui surviennent automatiquement chez ces blessés.

On procédera ainsi: traitement neurochirurgicale pour rémédier à la pression exercée sur la moelle épinière et pour soulager la douleur et la spasticité; procédés orthopédiques poûr corriger et stabiliser; physiotherapie pour fortifier les muscles, les os et les articulations; opérations urologiques pour restaurer le contrôle volontaire de la vessie urinarie; examens urologiques pour calculs et infections; opérations plastiques pour ulcus decubitus; diète soignée; programme pour surmonter le dommage psychique.

Les différences entre le traitement des paralysies spastiques et celui des paralysies flaccides sont discutées. Pour réussir, il est nécessaire de posséder des connaissances spéciales et d'être prêt à consacrer de longues heures de travail de la part de ceux qui soignent le malade et de détermination de la part du malade de recouvrer dans la mesure du possible le maximum de résultats.

## RESUMEN

Los autores se hallan en condiciones de informar, con toda autoridad, sobre el gran avance experimentado por el tratamiento y reacondicionamiento de las heridas en la médula espinal en el transcurso de la guerra. Al hablar sobre el tratamiento necesario que ha de capacitar a los enfermos para llevar una vida relativamente normal, plantean la necesidad de co-ordinar diversas especialidades, tales como la ortopedia, cirugía general y plástica, psicología, psiquiatría, terapéutica física y entrenamiento oral.

Se pone de relieve la importancia de un tratamiento inmediato debido a las condiciones que automáticamente sobrevienen de dichas lesiones, y se sumarizan, como sigue, los pasos necesarios del tratamiento: (1) Procedimientos neoroquirúrgicos para aliviar la presión sobre la médula espinal y aliviar el dolor y la incapacidad espasmódica; (2) Ortopedia correctiva y medidas de estabilización; (3) Práctica fisioterápica para fortalecer los músculos, huesos y articulaciones; (4) Operaciones urológicas para restaurar el control voluntario sobre la vejiga; (5) Exámenes urológicos para buscar piedras e infecciones; (6) Operaciones plásticas de las llagas producidas por la permanencia en la cama; (7) Estricta atención sobre la dieta y la nutrición; (8) Y por último, un programa de reconocimiento para acabar con los desperfectos físicos.

Se apuntan las diferencias en el tratamiento de las parálisis flácidas y espasmódicas. Los autores hacen hicapié, especialmente, en que él éxito de tal programa de reacondicionamiento exige un conocimiento especial, mucha práctica y largas horas de duro trabajo en la parte que se refiere al tratamiento del paciente, así como la decisión del enfermo de bastarse a sí mismo siempre que sea posible.

## Свод.

Авторы могут дать авторитетный доклад о прогрессе во время войны лечения и реабилитации при поражениях спинного мозга. Рассматривая условия, нужные для восстановления более или менее нормальной жизни этих больных, они подчеркивают необходимость координации разных специальностей: ортопедической, общей и пластической хирургии, психологии, психиатрии, физической терапии и тренировки языка

Подчеркнута важность немедленного лечения, потому что на почве этих повреждений автоматично возникают многие трудные условия Дан следующий порядок мер неврохирургические меры для облегчения давления на спинной мозг и для облегчения боли и спастичности, ортопедические исправляющие и стабилизирующие меры, квалифицированная физикотерапия для укрепления мышц, костей и суставов, урологические операции для ресторации контроля мочевого пузыря, урологические исследования возможности камней и инфекции, пластика пролежней, внимание к диэте и питанию и, наконец, реабилитационная программа с целью преодолеть психические повреждения Указана разница в лечении вялых и спастических параличей

Авторы особенно подчеркивают, что успех такой реабилитационной программы требует специальное знание, квалификацию и долгие часы работы со стороны тех, кто лечит больных, а также и твердую решимость со стороны самого больного сделаться насколько возможно самостоятельным

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## IMPENDING DEATH UNDER ANESTHESIA

*(Continued from page 10)*

de una jeringuilla o jeringuillas dentro del tuétano del hueso mejor que la aplicación de la cánula a una vena Su propia experiencia en el uso de la troca y cánula de punción externa, le han dado resultados de gran éxito

### Свод

Автор описывает методику сердечного массажа в случаях белой или синей асфиксии с остановкой сердца при общей анестезии Он считает этот способ оживления первым, а не последним средством На основании своего опыта он приходит к заключению, что, если массаж сердца применен в первую минуту с половиной после того как сердце прекратилось биться, то шансы на восстановление его действия гораздо лучше, чем если больше времени протекло. Он дает три успешные последующие случаи, в которых это правило было применено Укол желудочка сделан в первые 45 секунд по прекращении сердечного действия, и если результата нет, то немедленно применяется сердечный массаж

Промежуток времени в случаях цианозной асфиксии является более длинным, так как мозг не лишен крови В одном доложенном случае промежуток остановки сердца был, по крайней мере, 12 минут, результат был успешный

Он также обсуждает острый вазомоторный коллапс и рекомендует ин'екцию жидкости при помощи шприца в костяной мозг вместо канюлизации вены Его опыт в применении груднокостного троакара и канюлы был очень успешным

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munion between the grafts and the recipient sites.

6. Physiotherapy had to be employed after bone grafting in order to maintain the physiological integrity of the extremity and to realize good functional rehabilitation.

The assigning of these cases to the plastic surgeon became expedient for obvious reasons: first, the extensiveness of the injury destroyed all the conditions necessary for healing, as well as all the elements necessary for successful bone grafting; secondly, because the orthopedic surgeon by training and experience was not prepared to cope with such extreme losses and distortions of soft tissues.

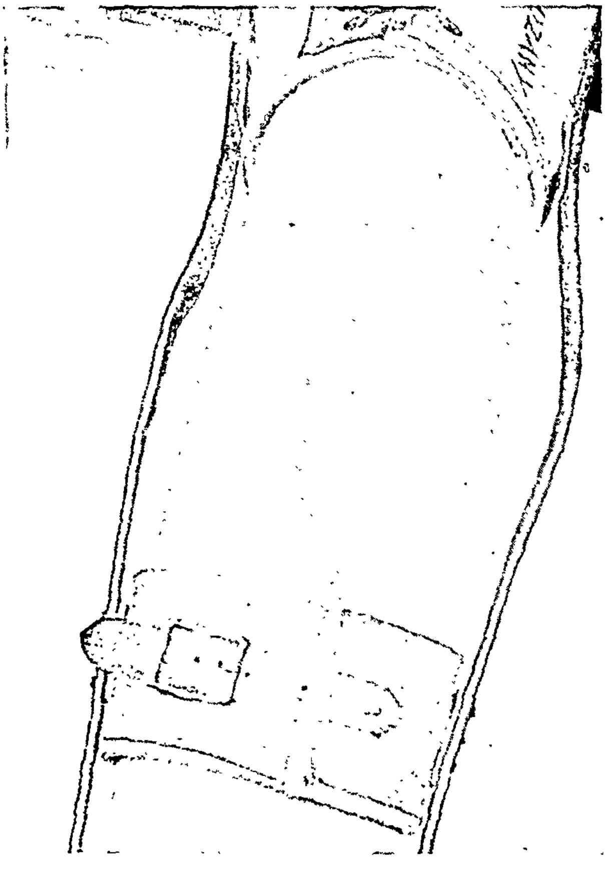

Fig. 4. Example of free grafting of original wound, to make a "closed" out of an "open" case. This procedure also obviates much of the fibrosis and distortion of tissues collateral to the wound.

The *plastic procedures* employed in the remedying of these injuries fell into two main groups:

1. The free grafting of the open wound, to expedite healing and to make a closed out of an open case. (Fig. 4.)
2. The reconstruction or reconstitution of the needed soft tissues.

Cases thus treated became a much easier nursing problem. This was of paramount importance due to the relative shortage of nurses in the army.

The reconstruction or reconstitution of the lost tissues was a more difficult problem. It involved the creation of massive grafts, too frequently when other parts of the patient's body most desirable for such grafts were also mutilated, and the transportation and implantation of these compound grafts into the wound.

The methods employed in general were the use of the single pedicle flap, sometimes referred to as the Italian method of repair, the French sliding flap, or the Filatov-Gillies tube.

The source of the surgeon's training, experience and, sometimes, prejudice, determined the choice of method. Many plastic surgeons, stimulated by the apparent ease and efficiency of the single pedicle flap method, were tempted into repairing practically all leg defects in this way. Others of us very early shied away from this method of repair for reasons to be given presently, and favored the tube graft or other methods which had to be modified to suit the exigencies of war surgery.

As a matter of fact, therein lies the crux of the whole matter of repair and rehabilitation of a severely wounded extremity. These two methods, the flap and the tube, are the foundation of all radical plastic repair, yet they are basically two entirely different types of surgery.

It is the purpose of this paper to draw a comparison between these two methods, and to cite others, in the hope that a sound and effective procedure may be generally established, in anticipation of such problems in civilian practice or, I regret to conjure, another possible war.

Let us then compare the virtues and shortcomings of the two methods. (Fig. 5.)

As mentioned earlier, there seemed to be a general preference for the use of the pedicle flap because it seemed easier of construction and management and was thought to give more expeditious results. The tube graft was looked upon as a protracted method of repair which did not ultimately give any better results than the flap.

I cannot entirely subscribe to either one of the foregoing opinions. The number of surgical innings necessary in the grafting of a given defect via the tube is four. The same invariably holds true for the single pedicle flap. Whereas an entire tube can be made in one stage, it usually takes at least two surgical innings, as I call them, to complete a transferable flap; since delay is unavoidable if adequate circulation to the distal end is to be assured. Only two of the three sides of a flap can safely be incised at the first, immediately followed by undermining. Incision of the third boundary of the flap is a separate procedure, usually done two or three weeks after the first.

True, complete immediate transfer of a mass of single pedunculated tissue can and has been done often by many of us, but it entails considerable risk in the lower extremity. It most certainly cannot be held out as a promising routine.

Fig 5

COMPARATIVE STUDY OF CERTAIN METHODS OF DERMOGRAFTING

| Type of Graft | Anesthetic | Minimum No. of Surgical Innings | Minimal Reconstruction Time—(Days) | Type of Surgery | Splinting Necessity | Complications | | | Tissue Cost | | | Economics | | Results | | | | Psychological | Clinical Rating—% |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Pedicle % | Graft % | Late % | Pedicle | Donor Site | Other | Hospital Time | Expense | Anatomic | Functional | Collateral Physiol | Esthetic | | |
| Single Peduncle Flap | L<br>Sp<br>S P | 4 | 50 | Open | Max | 17 | 14.5 | 5.9 | Mod | Max | Mod | Max | Max | Fair | Good | Fair | Good | Fair | 70 |
| Tube | Gen<br>Sp | 4 | 66 | Closed | Opt | 5 | 3.5 | 2 | Max | Min | Min | Mod | Mod | Good | Exc | Good | Good | Good | 80 |
| "Pincushion Flap | Gen<br>Sp | 3 | 44 | Closed | Opt | 3 | 1.5 | 0.5 | Mod | Mod | Mod | Mod | Mod | Good | Exc | Good | Good | Good | 85 |
| Double Peduncle Flap | L<br>Sp | 1 | 18 | Closed | Min | 1.5 | 1.5 | 1 | Min | Negl | Min | Min | Min | Exc | Exc. | Exc | Exc | Exc | 94 |
| French Flap | L<br>Sp | 1 | 14 | Closed | Min * | 2 | 1.5 | 0.5 | Min | Negl | Min | Min | Min | Exc. | Exc | Exc. | Exc | Exc | 100 |

L = local
Sp = spinal

S. P = Sod Pent
Max = Maximal

Opt = Optimal
Mod = Moderate

Negl = Negligible
Min = Minimal

"Other" under 'Tissue Cost" refers to need for split grafts for donor area
'Psychological considers effect of procedure on patient morale

complement for fixation can also be eluted from erythrocytes by heating at 56°C (12–16).

Because antibody could not be demonstrated in eluates from cells sensitized *in vivo* or *in vitro* in the serum of case 2, the elution technique did not help to clarify the nature of the "cell antibody" detected in the direct Coombs test.

### *Identification of PCH serum factor reacting with antiglobulin serum as PCH hemolysin*

The use of antiglobulin serum to detect and measure PCH antibody indicated that agglutination in the indirect Coombs tests was dependent on the same serum factor that produced hemolysis in the Donath-Landsteiner reaction (4). The indications that this is true are summarized as follows:

*1*) Neither Coombs antibody nor hemolysin was adsorbed at 10–12°C; both required 4°C for adsorption.

*2*) Coombs antibody and hemolysin were adsorbed simultaneously.

*3*) The titers of Coombs antibody and hemolysin were the same (4).

*4*) Both withstood heating at 62°C for 30 minutes.

*5*) There was a reciprocal relationship between the amount of complement present and both the amount of Coombs antibody fixed and the hemolysin titer (4).

*6*) Both, as will be indicated, are in the same serum fraction.

### *The separation and characterization of the PCH antibody*

Characterization of PCH antibody necessitated separation of the antibody from other serum components. The possibility existed that the patient's serum might contribute C′1 and C′3 activity or an abnormal component which would complete the hemolytic reaction in the presence of C′2 and C′4. Fraction PI contained C′1,C′3, and C′4 but did not contain any PCH antibody (Table III). The precipitates formed when Fraction SI was dialyzed did not contain PCH antibody nor did they contain any of the components of complement. On the basis of these studies, which showed that the PCH antibody was not associated with C′1, C′3, and C′4, nor with the euglobulins which are insoluble between pH 5.0 and 7.0, the possible association of PCH antibody with C′2 or with the gamma globulins was next investigated.

The precipitates II + III, IV and V were examined for their PCH antibody content (Table III); II + III was the only fraction which contained PCH antibody. II + III prepared in the manner described above did not contain any of the known components of complement, and it was obvious that the PCH antibody was not associated with complement in human plasma.

TABLE III

*Titration of PCH antibody in various fractions of serum*

| Serum fraction | Antibody titration | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Hemolysin | | | | | Indirect Coombs (b) | | | | | |
| | Undiluted | 2 | 4 | 8 | 16 | Undiluted | 2 | 4 | 8 | 16 | 32 |
| Original plasma | +++ | ++ | + | 0 | 0 | +++ | ++ | ++ | 0 | 0 | 0 |
| PI* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| II+III*† | 0 | + | ++ | + | 0 | +++ | +++ | +++ | ++ | ++ | 0 |
| IV* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| V* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| II‡ | +++ | ++ | ++ | + | 0 | ++++ | ++++ | ++++ | +++ | + | 0 |
| III-1‡ | ++ | + | 0 | 0 | 0 | ++++ | +++ | 0 | 0 | 0 | 0 |
| II E§ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| II S§ | ++ | + | 0 | 0 | 0 | ++++ | +++ | ++ | 0 | 0 | 0 |

* Fraction concentrated five times with respect to original plasma.

† II+III anticomplementary in dilutions through 1–4.

‡ Fraction concentrated four times with respect to original plasma.

§ Fraction concentrated two and one-half times with respect to original plasma.

When II + III was separated into water soluble (II + IIIS) and water insoluble (II + IIIE) fractions, PCH antibody was found in both of these fractions. II + IIIS and II + IIIE were therefore combined (II + III) and fractionated into II and III − 1. Practically all of the PCH activity was found in Fraction II. Small amounts were found in III − 1. Electrophoretic analysis of Fraction II revealed that it was entirely homogeneous with a mobility identical to that of the gamma globulins.

When Fraction II was separated into water soluble (IIS) and water insoluble (IIE) fractions, all of the PCH antibody was found in IIS indicating that this antibody is associated with water soluble gamma globulins and resembles most other human antibodies in this respect (9).

*Comment:* It is apparent that none of the known components of complement are associated with PCH antibody. In fact, the antibody is a gamma globulin.

PCH antibody in Fraction II + III behaved as though it was a euglobulin but actually showed its true pseudoglobulin character in Fraction II. This discrepancy may be due to interactions occurring between PCH antibody and other globulins in II + III. The removal of alpha and beta globulins allowed the antibody to exhibit its solubility in water.

While most of the PCH antibody was present in II, small amounts were found in III − 1. The gamma globulin originally present in II + III was distributed in the proportion of 80 per cent in II and 20 per cent in III − 1. Thus, the activity found in III − 1 may be accounted for by the presence of small amounts of gamma globulin in this fraction. When Fraction II was separated into euglobulins and pseudoglobulins, all of the antibody activity was associated with the water soluble gamma globulins (IIS). Fraction IIS contains other antibodies as well as the PCH antibody (9), and it is hoped that PCH antibody can be separated from IIS by adsorption and elution. The possibility still exists that a serum factor independent of complement and antibody is concerned in the hemolysis of paroxysmal cold hemoglobinuria. Complete elucidation of this problem must await studies with pure components of complement and pure PCH antibody.

## SUMMARY

Erythrocytes from a patient with paroxysmal cold hemoglobinuria (PCH) were found to be agglutinable in antiglobulin serum (direct Coombs test). Although these erythrocytes had been "sensitized" *in vivo,* they were not hemolyzed when warmed with complement. The cells, however, were hemolyzed, both *in vivo* when the patient was chilled, and *in vitro* when they were chilled and warmed in PCH serum.

The patient's cells were no longer agglutinable in antiglobulin serum six hours after *in vivo* hemolysis. No concomitant change in either serum antibody or complement level was detected. Further characterization of the "cell antibody" responsible for agglutination in the direct Coombs test was not possible since the factor could not be demonstrated in eluates from the patient's erythrocytes.

The behavior of the abnormal antibody present in the sera of two patients with PCH was studied by using antiglobulin serum (indirect Coombs test) as well as the Donath-Landsteiner reaction. Antibody adsorbed *in vitro* from serum with a high antibody titer could be eluted from erythrocytes by heating the cells at 56°C.

The PCH serum factor which reacts with antiglobulin serum was identified as the PCH hemolysin.

Fractionation of PCH serum in alcohol-water systems revealed that the PCH antibody is a water soluble (pseudoglobulin) gamma globulin and resembles most other human antibodies in this respect.

## ACKNOWLEDGMENT

The technical assistance of Miss Marjorie Newton and Miss Sylvia Rowihab is gratefully acknowledged.

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# DIRECT ACTION OF VITAMIN $B_{12}$ UPON HUMAN BONE MARROW

## THE EFFECT OF INSTILLATIONS OF VITAMIN $B_{12}$ AND FOLIC ACID INTO THE BONE MARROW AS STUDIED BY NUCLEIC ACID STAINING TECHNIQUES[1, 2]

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(Submitted for publication July 5, 1950; accepted, October 10, 1950)

Folic acid and vitamin $B_{12}$ are involved in chemical reactions which lead to the formation of nucleic acids in living cells. These relationships are amply demonstrated by studies in bacterial (1–5), animal (6), and human (7) metabolism. It appears likely that folic acid is essential to the formation of various purines and pyrimidines such as thymine from endogenous sources of carbon and amino nitrogen as well as to the interconversion of these substances or of their ribosides (8). Vitamin $B_{12}$, on the other hand, appears to be active in the formation of ribosides, such as thymidine, from these purines and pyrimidines. Folic acid and vitamin $B_{12}$, therefore, appear to be active at different stages of a chemical chain reaction which leads to the formation of nucleic acids.

In this paper, we wish to report a method of direct instillation of test substances into human bone marrow cavities. By such a method, observations may be made on the effect of various substances to be tested on marrow cells in their natural environment. By the use of this method, evidence has been obtained that vitamin $B_{12}$ can be utilized unchanged by the megaloblasts and early erythroblasts of patients with pernicious anemia in relapse. On the other hand, folic acid has no such direct effect. Methyl green-pyronin stain applied to the erythroblasts of patients with pernicious anemia in relapse before and after the instillation of vitamin $B_{12}$ into the bone marrow indicates that vitamin $B_{12}$ corrects a qualitative abnormality of the cytoplasmic ribonucleic acid of these cells. Folic acid has a similar effect only after oral or parenteral administration and is presumably activated by enzymatic activity elsewhere in the body. These data offer additional evidence relating vitamin $B_{12}$ and folic acid to nucleic acid metabolism.

### MATERIAL AND METHODS

Injections into the marrow cavities of the iliac crests of six persons with pernicious anemia in relapse were performed as follows: three received 1 microgram of crystalline vitamin $B_{12}$[3] and three received 1 or 2 mgs. of folic acid.[4] In each instance, the material was injected slowly in 1 c.c. of solution after aspiration of 2–3 c.c. of marrow and blood. Moderate pain accompanied the injection and was diminished by slowing the rate of instillation. Forty-eight hours later, bone marrow was again aspirated from the exact site of previous instillation and also from the opposite iliac crest. The site of instillation was easily located by using the trauma produced by the earlier puncture as a landmark. In one case, marrow was aspirated also from sites 4 and 8 centimeters distant from the site of instillation of the vitamin $B_{12}$. Coverslip preparations were made of each marrow specimen obtained and stained *1)* with Wright-Giemsa stain for differential cell counts, *2)* with methyl green and pyronin B (9) for demonstration of cytoplasmic ribonucleic acid, and *3)* by a Stowell's modification of the Feulgen method (10) for demonstration of desoxyribonucleic acid. The methyl green-pyronin stain was prepared by mixing three parts of a 1 per cent solution of methyl green[5] containing 0.25 per cent liquefied phenol

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[1] This work was aided by a grant from the Robert Gould Research Foundation.

[2] Read in part before the Forty-second Annual Meeting of the American Society for Clinical Investigation, Atlantic City, N.J., May 1, 1950.

[3] The material used was "Cobione," supplied by Merck and Co., Inc., Rahway, N.J., and provided in 1 c.c. ampules containing 10 micrograms of crystalline vitamine $B_{12}$ per c.c. Appropriate dilutions were made for this study with physiologic saline solution.

[4] The material used was "Folvite," supplied as a powder by the Lederle division of the American Cyanamid Corp., Pearl River, N.Y. Solutions containing 1 or 2 mgs. of folic acid per c.c. were made in distilled water containing sodium bicarbonate sufficient for a pH of approximately 7.5.

[5] Methyl green and pyronin B were obtained from the National Aniline Division, Allied Chemical and Dye Corp., New York, N.Y. The stains used were certified by the Biological Stain Commission.

TABLE I

*Nucleated erythroid cells in bone marrow before and after local instillation of vitamin $B_{12}$*

| | | Case I | | | Case II | | | Case III | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Before vit. $B_{12}$ | After vit $B_{12}$ | Opposite ilium after vit. $B_{12}$ | Before vit. $B_{12}$ | After vit $B_{12}$ | Opposite ilium after vit $B_{12}$ | Before vit. $B_{12}$ | After vit. $B_{12}$ | Opposite ilium after vit. $B_{12}$ |
| | | | *1 gamma* | | | *1 gamma* | | | *1 gamma* | |
| Megaloblasts | Per 100 WBC | 16.0 | 3.5 | 11.0 | 13.5 | 1.5 | 17.5 | 15.5 | 3.0 | 13.0 |
| Early erythroblasts | | 32.0 | 15.5 | 32.5 | 26.5 | 26.5 | 28.5 | 35.5 | 26.5 | 37.5 |
| Late erythroblasts | | 18.0 | 25.5 | 14.5 | 15.0 | 35.0 | 18.5 | 12.0 | 28.0 | 20.5 |
| Normoblasts | | 43.0 | 100.5 | 43.5 | 40.5 | 90.5 | 48.0 | 24.5 | 87.5 | 21.5 |

to one part of a similarly prepared solution of pyronin B.[5] Coverslip marrow films were stained for ten minutes following fixation for one minute in 95 per cent alcohol.

Films of bone marrow from a patient with erythroid hyperplasia due to chronic blood loss and from a normal person were treated in the same manner and served as controls. The red color of the cytoplasm produced by the pyronin stain indicated the presence of ribonucleic acid since this color could not be developed following incubation of films for three hours at 60° C. in a 0.1 per cent solution of crystalline ribonuclease[6] buffered with sodium barbital to a pH of 6.75 (11).

## RESULTS

*Effect of local marrow instillation of vitamin $B_{12}$ on erythrocyte maturation:* Table I is a chart of differential counts of nucleated cells of the erythroid series in bone marrow from three patients with pernicious anemia in relapse before and after the instillation of vitamin $B_{12}$ into the marrow cavity. Stimulation of erythroid maturation is shown in all three instances by a decrease in the number of megaloblasts and an increase in normoblasts in the specimens obtained 48 hours after the local marrow instillation of the Vitamin $B_{12}$ (see Figures 1 and 2). The marrow obtained from the opposite iliac crest was unaffected, indicating that the stimulation induced by vitamin $B_{12}$ was local when the amount instilled was 1 microgram. However, the local marrow instillations of 15 micrograms resulted in a systemic response. In the one instance that marrow was aspirated 4 and

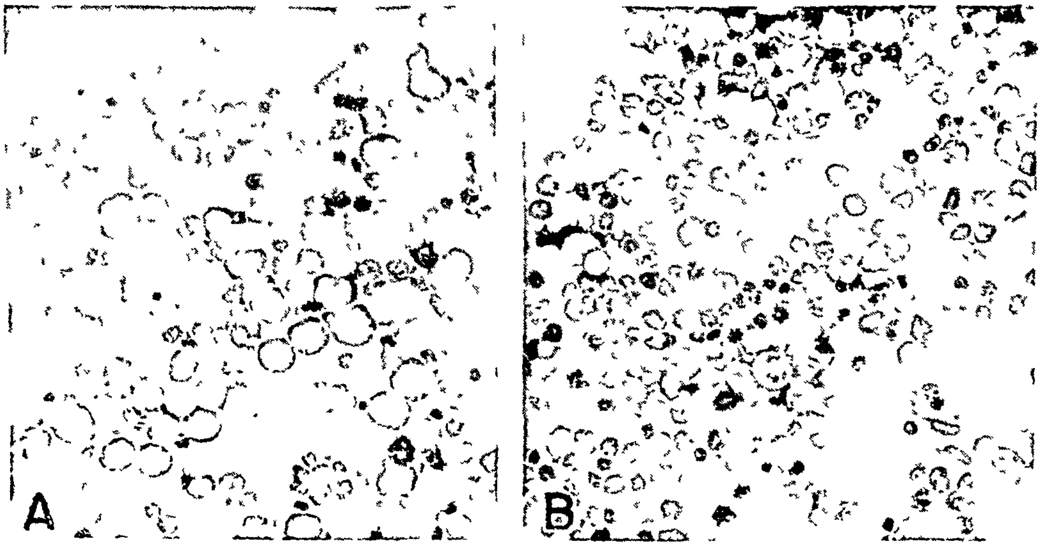

FIG. 1. THE EFFECT OF LOCAL MARROW INSTILLATION OF VITAMIN $B_{12}$ ON WRIGHT-GIEMSA STAINED MARROW FILMS

A megaloblastic maturation arrest characteristic of pernicious anemia is seen in (A). A shift to a normoblastic maturation level at the injection site 48 hours after instillation of vitamin $B_{12}$ is seen in (B). At this time, the marrow obtained from the opposite ilium was identical with (A). (× 320)

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[6] Crystalline ribonuclease was provided by the Worthington Biochemical Laboratory, Freehold, N. J.

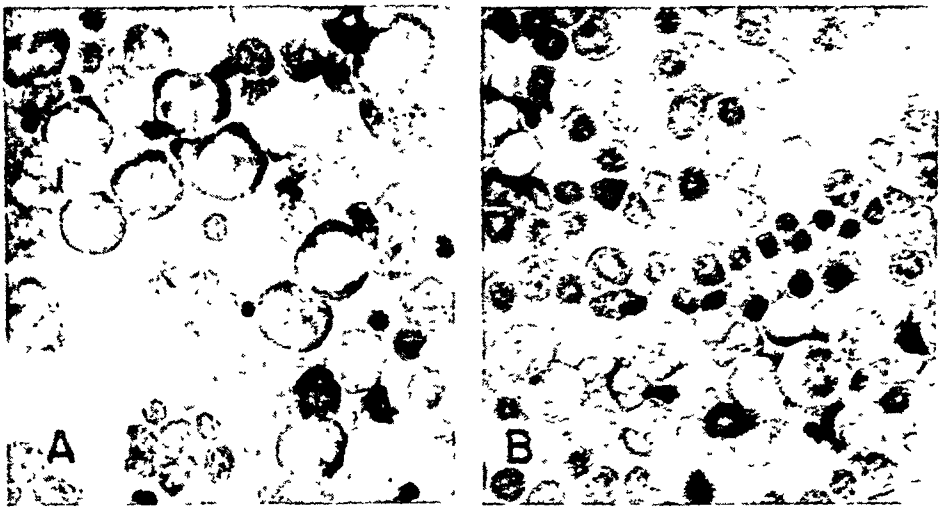

*FIG. 2.* MAGNIFICATION OF CELLS FROM FIGURE 1

(A) Megaloblasts of pernicious anemia. (B) Normoblasts. Wright-Giemsa stain. (× 650)

8 centimeters from the site of injection, the stimulating effect decreased as the distance increased.

*Effect of local marrow instillation of folic acid on erythrocyte maturation:* Table II is a chart of differential counts of nucleated erythroid cells in bone marrow from three patients with pernicious anemia in relapse before and after the local marrow instillation of 1 or 2 mgs. of folic acid. It is apparent that there was no stimulation to erythroid maturation at the site of injection of folic acid. The counts done on marrow before and after injection and on marrow from the opposite ilium are all similar. Furthermore, no change in the appearance of Wright-Giemsa stained marrow films was noted after instillation of folic acid into the marrow cavity.

*Effect of local marrow instillation of vitamin $B_{12}$ and folic acid on the nucleic acid content of erythroid cells:* The character and distribution of ribonucleic acid in the cytoplasm of young erythroid cells were observed in methyl green-pyronin stained preparations of bone marrow from a patient with erythroid hyperplasia resulting from anemia of chronic blood loss. Study of such a preparation (Figure 3) reveals a diffuse and homogeneous distribution of the red granules throughout the cytoplasm of the early erythroblasts. In the later forms, they seem to become more condensed and clumped. Finally, during the normoblastic stage of the development of the erythrocyte, the red color, indicating cytoplasmic ribonucleic acid, disappears altogether.

Methyl green-pyronin stained marrow preparations from patients with pernicious anemia in relapse, one of which is presented in Figure 4-A, show that the distribution of the cytoplasmic ribonucleic acid is quite different in this disease. In these preparations, there is condensation and

TABLE II

*Nucleated erythroid cells in bone marrow before and after local instillation of folic acid*

| | | Case IV | | | Case V | | | Case VI | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Before folic acid | After folic acid | Opposite ilium after folic acid | Before folic acid | After folic acid | Opposite ilium after folic acid | Before folic acid | After folic acid | Opposite ilium after folic acid |
| | | | *1 mg.* | | | *1 mg.* | | | *2 mgs.* | |
| Megaloblasts | Per 100 WBC | 6.0 | 7.0 | 3.5 | 7.5 | 8.5 | 5.5 | 13.5 | 15.0 | 11.0 |
| Early erythroblasts | | 10.5 | 8.0 | 6.5 | 25.5 | 23.0 | 16.5 | 22.0 | 19.0 | 18.5 |
| Late erythroblasts | | 7.0 | 3.0 | 4.5 | 17.5 | 21.5 | 16.0 | 15.0 | 12.0 | 10.5 |
| Normoblasts | | 24.5 | 17.5 | 27.0 | 21.5 | 29.0 | 31.0 | 20.0 | 25.0 | 27.5 |

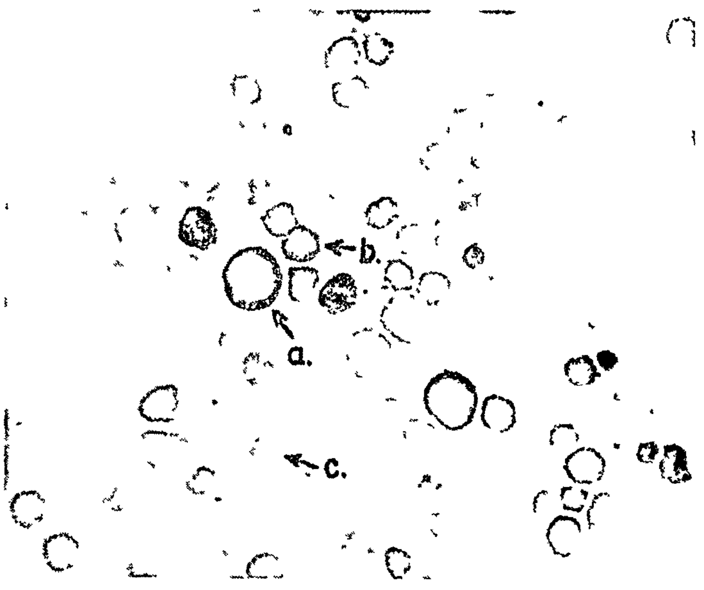

Fig 3 A Film of Marrow from a Patient with Chronic Blood Loss Anemia Stained with Methyl Green and Pyronin B

Cytoplasmic ribonucleic acid stains red; nuclear structures stain green a = early erythroblast; b = late erythroblast; c = normoblast. (× 650)

clumping of the red granules in the cytoplasm of the young cells of the erythroid series. In similarly stained marrow smears (Figure 4-B) after local marrow instillation of vitamin B$_{12}$, there is a change in the appearance of the cytoplasmic ribonucleic acid of these early erythroid cells. It becomes more diffuse and homogeneous; and, in general, it assumes the characteristics of that observed in early erythroblasts from a patient with anemia due to chronic blood loss.

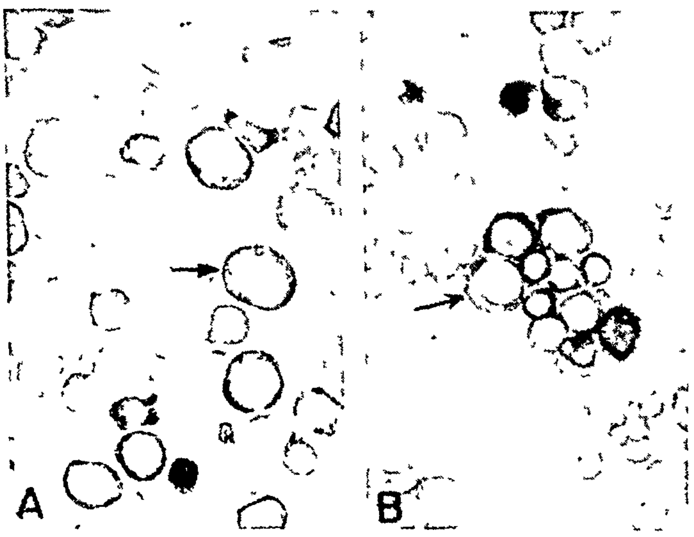

Fig 4 The Effect of Local Marrow Instillation of Vitamin B$_{12}$ on Methyl Green-Pyronin B Stained Marrow Films

In (A) arrow points to early erythroblast in marrow from patient with pernicious anemia in relapse In (B) arrow points to early erythroblast in marrow obtained 48 hours later from the injection site of vitamin B$_{12}$ (× 650)

No change in the clumped cytoplasmic ribonucleic acid was noted 48 hours after the local marrow *instillation* of folic acid. However, when folic acid was given orally or intramuscularly in therapeutic doses, the cytoplasmic ribonucleic acid became homogeneous and of more normal appearance.

The appearance of desoxyribonucleic acid stained by Stowell's modification of the Feulgen method was not altered in the preparations obtained after the instillation either of vitamin B$_{12}$ or of folic acid. Furthermore, the desoxyribonucleic acid in erythroblasts from persons with pernicious anemia in relapse had the same appearance as that from a person with anemia of chronic blood loss.

## DISCUSSION

The normal maturation of erythroblasts in the bone marrow of persons with pernicious anemia in relapse observed at the site of instillation of vitamin B$_{12}$ but not in marrow from the opposite ilium demonstrates that vitamin B$_{12}$ can be utilized directly by bone marrow cells without alteration by any other organ or tissue. Therefore, if vitamin B$_{12}$ and extrinsic factor of food are the same substance, as seems probable, this experiment provides direct evidence that extrinsic factor need not be chemically changed by intrinsic factor or by the liver before becoming active for erythrocyte maturation. This, of course, is not a new concept. Studies of Berk and his associates (12) on hematologic responses in persons with pernicious anemia, who received vitamin B$_{12}$ and refined liver extract orally along with normal human gastric juice, suggest that vitamin B$_{12}$ (presumably extrinsic factor of food) and the antipernicious anemia principle of liver are identical or closely related compounds. After observing the magnitude of fecal elimination of vitamin B$_{12}$ in patients with untreated pernicious anemia and the hematologic responses in similar patients obtained by the administration of 10 per cent alcoholic extracts of beef muscle, they concluded that ". . . the function of the intrinsic factor of normal human gastric juice is to facilitate the absorption by the intestine of vitamin $B_{12}$ . . . rather than to react with the extrinsic factor as hitherto assumed."

On the other hand, in our experiment, folic acid was not utilized unchanged within 48 hours after instillation of the substance into the marrow cavity. Observations by others provide further data that folic acid, acting as a substrate, in some way must be altered before becoming active in hematopoiesis. Norris and Majnarich have reported that folic acid has no effect on hematopoiesis as observed by an *in vitro* rabbit bone marrow culture technique (13). However, after this substance was incubated with milk xanthine oxidase, liver homogenate, or extract of rat gastric mucosa, it became hematopoietically active as demonstrated by this method (14). Tove and Elvejhem (15) have reported the presence of a substance in methanol extract of liver which has a sparing effect on folic acid in the metabolism of mink. Furthermore, this substance protected these animals from folic acid deficiency produced by feeding them 7-methyl folic acid.

Further stimulation to the study of the mode of action of folic acid in hematopoiesis was provided by the concentration by Sauberlich (16) of a substance in rat urine which is a growth factor for *Leuconostoc citrovorum* 8081. Studies on this factor, termed the "citrovorum factor," have demonstrated that *1*) the urinary excretion of the substance is increased by oral administration of folic acid both in rats (17) and in human beings (17,18), and *2*) the substance counteracts the toxicity of aminopterin as measured by growth of *L. citrovorum* (17). Data are not yet available on the hematopoietic activity of this substance in erythrocyte maturation factor deficiency anemia. Preliminary studies in this laboratory indicate that the local marrow instillation of four million units of "citrovorum factor" does not result in stimulation of erythrocyte maturation in persons with pernicious anemia in relapse.

The changes observed in the cytoplasmic ribonucleic acid of erythroid precursor cells in marrow from subjects with pernicious anemia in relapse as compared with those from persons with anemia of chronic blood loss provide data linking erythrocyte maturation factor deficiency anemia to abnormalities in nucleoprotein metabolism. Thorell, in 1947 (19), correlated, by cytochemical and ultraviolet microphotographic studies, endocellular growth process in hematopoiesis with the presence of ribonucleic acid in the granulocytic and erythroid precursor cells in bone marrow. Briefly, he found that growth and new cellular protein formation during hematopoiesis are associated with high concentrations of ribonucleic acid in the cytoplasm and the nucleolus of these cells; as growth activity declines, the concentrations of ribonucleic acid decrease proportionately. In normal erythropoiesis, hemoglobin synthesis does not begin until the ribonucleic acid metabolism is completed; after this, the synthesis of hemoglobin proceeds rapidly. In pernicious anemia, there is an abnormality in these relationships of growth and differentiation; in this disease, hemoglobin synthesis begins while the ribose polynucleotide content of the cell is still high. The apparent correction of this abnormality observed in our study after the local marrow instillation of vitamin $B_{12}$ provides additional data linking vitamin $B_{12}$ and nucleoprotein metabolism.

## SUMMARY AND CONCLUSIONS

1. A method of direct instillation of test substances into human bone marrow cavities is described.

2. Vitamin $B_{12}$ can be utilized locally by the bone marrow cells and corrects a qualitative abnormality in cellular ribonucleic acid in persons with pernicious anemia in relapse. It need not be altered by stomach or liver to exert this effect.

3. Folic acid is not utilized locally by bone marrow cells within 48 hours of instillation into the marrow cavity; but when given orally or parenterally, it has the same cytologic and cytochemical effects as vitamin $B_{12}$. It is probable that folic acid must be converted to an active hematopoietic substance by enzymatic activity elsewhere in the body.

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# NEURAL MECHANISMS INVOLVED IN ITCH, "ITCHY SKIN," AND TICKLE SENSATIONS

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(Submitted for publication August 4, 1950; accepted, October 10, 1950)

Available evidence suggests that the sensation of itching is closely related to that of pain (1). Titchener (2) observed that when the skin was explored with a fine hair, well-defined points were found which gave rise to itching when the intensity of stimulation was low, and to pain on stronger stimulation. Bishop (3) found that itching resulted from repetitive low intensity electrical stimulation of pain spots in the skin. Lewis, Grant and Marvin (4) pointed out that noxious stimuli, if their intensity be decreased, can be made to produce itching instead of pain. Förster (5) and Bickford (6) reported that in patients who had undergone section of the lateral spinothalamic tracts, thus abolishing perception of cutaneous pain but not of touch, itch was also abolished. Bickford also observed this combination of sensory changes in two patients with syringomyelia. Ehrenwald and Königstein (7), however, stated that they had found two cases of syringomyelia with loss of pain but preservation of touch and itch, and one in which pain was intact but touch and itch absent. McMurray (8) and Kunkle and Chapman (9) found that individuals who were constitutionally insensitive to pain were likewise insensitive to itching, but they experienced touch sensation. Bickford observed that itching and pain disappeared at the same time when a cutaneous nerve was anesthetized. Hardy, Wolff and Goodell (10) observed that in addition to feelings of warmth, occasional itching resulted from sustained thermal irradiation at an intensity not quite strong enough to induce pain. Thöle (11) found during spinal anaesthesia, as the sensitivity of the skin to painful stimuli decreased, that stimuli which initially induced pain later induced itching and finally no sensation at all.

In the present investigation many of the experiments described by Goldscheider (12), Bickford, and Lewis and associates have been repeated. Additional experiments were designed to illuminate further the neural mechanisms involved in itch, "itchy skin" and tickle sensation.

## ITCH

### *Subjects and methods*

The subjects were healthy adults, chiefly the authors, although other individuals participated from time to time. Itching was elicited by the application of cowhage to an area of skin approximately 1 cm. in diameter. Cowhage is the familiar "itch powder," consisting of the fine fibers or spicules of the plant *Mucuna pruriens.* It was found by trial that the itch so produced was indistinguishable from that following the bites of mosquitoes, or the intracutaneous injection of histamine. When an area of skin on the arm, hand, leg or back was used for experimental procedures the corresponding areas on the opposite side of the body served as control.

### *Description of sensation induced following application of cowhage to the skin*

Cowhage applied to an area of skin approximately 1 cm. in diameter induced, usually within 10–20 seconds, an intense itching sensation which had both burning and pricking qualities. It was localized but seemed to spread for a few millimeters beyond the borders of the stimulated area. These itch sensations sometimes merged into pain of a burning and pricking quality. The skin under the cowhage rapidly became red, and a flare sometimes spread into adjacent areas of skin. When the area involved was the volar surface of the forearm, the flare spread as far as 2 cm. proximally and distally and approximately ½ to 1 cm. laterally. Within a few minutes after application of the cowhage, the skin several centimeters proximally and distally around the itching area became hypoalgesic to pin prick, and around the edge of this zone was a narrow band of hyperalgesia to pin prick, approximately ½ cm. wide. The itching from cowhage spontaneously diminished after a few minutes and did not return until the cowhage was mechanically agitated; or until it was revived by stroking the surrounding skin with the finger tip or a blunt object.

When the cowhage applied to the skin produced an intense itching, the sensation sometimes eventually became indistinguishable from pain. Furthermore, occasionally cowhage on normal skin and in areas of primary or secondary hyperalgesia induced pain alone. Also, as mentioned above, a heat stimulus at an intensity just below that sufficient to elicit pain, elicited itching.

### *Peripheral Fiber Pathways Involved in Itch Sensation*

It has been demonstrated that stimulation of the human skin with a pin may give rise to two kinds of painful sensation. The first pain to be perceived after the application of the pin is described as "pricking" and is punctate, superficial and well localized. This has been referred to as "first" or "fast" pain. There is also a second, different type of pain, described as "burning," which is perceived as more diffuse and less superficial than the "pricking" pain. Since this "burning" pain is perceived after a slightly but definitely longer latent period than the "pricking" pain, it has been referred to as "second" or "slow" pain (13, 14). Although Lewis denied that these experiences constituted different qualities of cutaneous pain, others have recognized the qualitative difference. Most individuals can readily distinguish between the "pricking" and "burning" in cutaneous pain (15), especially if they are separately presented by the experimental methods described below.

The work of both Lewis and co-workers and Gasser and co-workers suggests that the "fast" pain is a function of myelinated fibers, the "slow" pain of unmyelinated ones (14).

#### *Experiment 1. Demonstration of an itching sensation with pricking quality*

In three subjects procaine hydrochloride 1% was infiltrated about a cutaneous nerve on the ventral aspect of the forearm. Within a few minutes there developed distal to the site of infiltration an area in which pin prick elicited only a sharp, superficial, well-localized pain of short latency—the "first" pain. The slower, diffuse and burning "second" pain was not felt.

Within this area of altered sensibility cowhage elicited itching which was sharp, pricking, superficial, and readily localized. There was no burning component. In areas where there was complete absence of pain sensitivity, but where light touch was still present, itching was not felt.

#### *Experiment 2. Demonstration of itching sensation with a burning quality*

*a*) In a series of six experiments on four subjects ischemia of the forearm was obtained by a pressure of 200 mm. Hg maintained around the upper arm by means of a sphygmomanometer cuff. After about 20 minutes of ischemia in the arm, a pin prick applied to the fingers and the hand elicited pain of long latency which was poorly localized, and burning in character. The "first" pain was absent, as was light touch.

Within these areas of altered sensibility the application of cowhage on a site approximately 1 cm. in diameter on the back of the hand resulted in itching which was different from that obtained in Experiment 1, in that it was diffuse and poorly localized, seemed to be a little distance beneath the surface of the skin, and was "burning" in quality.

However, in more proximal areas of the ischemic forearm, closer to the occluding cuff, where normal pain and touch sensation were retained, cowhage produced itching no different from that in skin of the opposite arm with blood and nerve supply intact.

*b*) The course of a branch of a superficial cutaneous nerve in the forearm was mapped through a distance of 4–6 cm. by means of faradic stimulation in two subjects. A soft lead tubing was applied over the nerve and held in place by tapes. Brine at a temperature of $-2°$ to $-4°$ C was circulated through it for 30 minutes, resulting in the progressive anesthetization of the area supplied by the nerve (16).

After 30 minutes, within this area of altered sensation "second" pain could be elicited by a pin point but "first" pain and light touch were absent. In these areas "burning" itch could be elicited by cowhage but not "pricking" itch.

### *Comment*

It was thus possible to separate the usual itch sensation, as it occurs after insect bites or the application of cowhage, into two components. These corresponded in quality to the two types of cutaneous pain, and the results of differential blocking of cutaneous nerves indicate that they are mediated by different fibers. It is suggested that "pricking" itch is carried by the myelinated fibers responsible for "first" pain, and that "burning" itch is carried by unmyelinated fibers responsible for the "second" pain. Since either kind of itch could occur in skin in which touch perception was absent, it seems probable that touch receptors and nerves are not involved in the perception of itching.

It might be objected that the sensory changes following obstruction of the circulation are due to interference of fluid movement which might modify the function of sensory end organs, rather than to any functional change in the nerves themselves.

Against this assumption is the evidence that normal sensation was retained in skin close to the occluding cuff, but still distal to the obstruction, where fluid movement was as much interfered with as in the more distal areas where sensory changes did occur.

It is not difficult, once the two sensory components of itching have been separately perceived, to identify them in spontaneously occurring itches or in itch induced by a heat stimulus, by intracutaneous puncture of histamine or by exposing the arm to multiple mosquito bites.

Since the sensations of itching and pain are apparently mediated by the same fiber pathways an attempt was made to ascertain in what way a stimulus which produces itching differs from one which produces pain. Hence, experiments to test the thesis that the stimuli are of the same kind but of lower intensity were designed.

*Experiment 3. Demonstration of lowered pain threshold in itching areas*

Several areas of skin of the volar surface of the forearm were blackened with India ink, as shown in Figure 1. Pain thresholds in all areas were measured by the thermal radiation method of Hardy, Wolff and Goodell (17). Cowhage was applied to the central area in the usual way. Pain thresholds were then measured repeatedly both when itching was present and in the intervals when it had spontaneously but temporarily ceased.

It was found that the pain threshold in all five areas was the same before cowhage was applied (190 ± 5 millicalories/sec./cm.$^2$). After itching started in the central area, however, the threshold in this zone was lowered (120 to 160 mc./sec./cm.$^2$), the lowest thresholds being found at the times of highest itch intensity. When itching temporarily ceased, the threshold returned to its initial level, and was lowered again on the resumption of itching. In the other areas the threshold remained constant or was slightly elevated to 210 ± 10 mc./sec./cm.$^2$ when the central area was itching. These observations were made in three series of experiments in each of two subjects. The application of the heat stimulus to the central area in itch free intervals was usually followed by recrudescence of the itching.

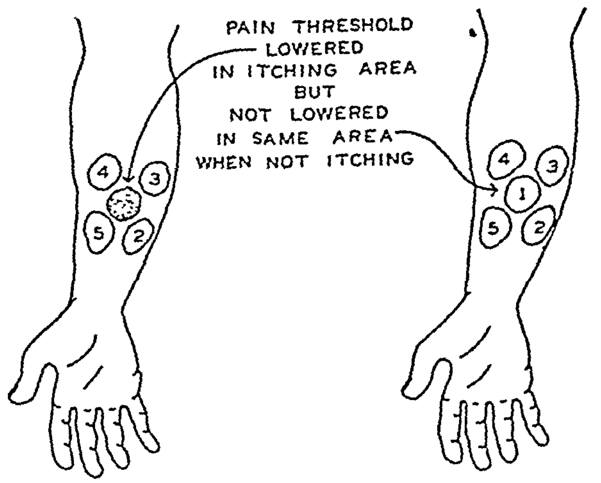
PAIN THRESHOLD LOWERED IN ITCHING AREA BUT NOT LOWERED IN SAME AREA WHEN NOT ITCHING
4
3
5
2
4
3
1
5
2

FIG. 1. LOWERING OF THE PAIN THRESHOLD IN AN AREA OF ITCHING SKIN

Cowhage was applied only to area 1. When itching occurred pain threshold was lowered in area 1, but not in adjacent areas 2, 3, 4, and 5.

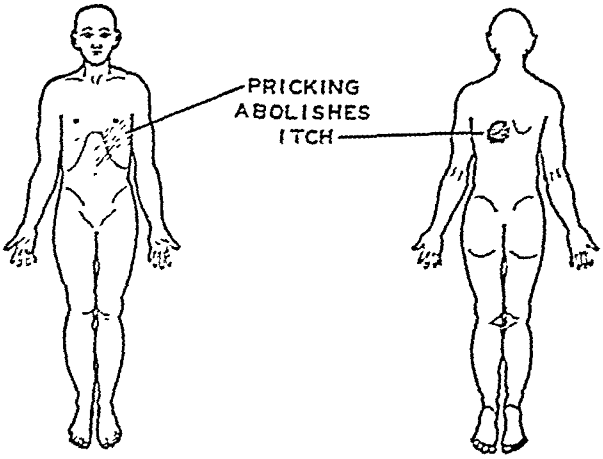

FIG. 2

Itching induced on the back by cowhage was abolished by painful pin pricks in the same dermatome on the anterior chest wall.

## *Comment*

These observations are consistent with the view that stimuli which give rise to itching activate pain endings in the skin at a stimulation intensity below the pain threshold. Less additional thermal energy is therefore required to produce definite pain sensation if itching is already occurring. Hence the pain threshold as measured is lowered.

## *Alterations in Central Excitatory Processes Relevant to Itching*

*Experiment 4. Demonstration that itch can be abolished by painful pin pricks in adjacent skin*

It was found in 20 subjects that when itching occurred spontaneously or when it was induced by cowhage, a pin pricked lightly several times in the zone of itching on the surrounding skin abolished the itching sometimes for long intervals. It usually returned after periods up to several minutes in duration, although the slight pain from the prick had completely faded within 10 to 15 seconds. This has been observed not only with itching due to cowhage, but also in the itching resulting from

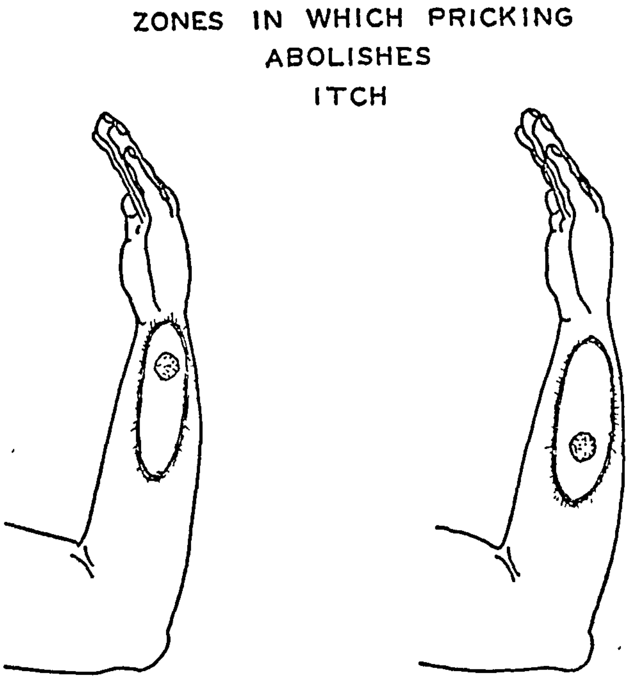

FIG. 3. ZONES IN WHICH PAINFUL PIN PRICKS ABOLISHED ITCHING INDUCED BY COWHAGE ON THE MEDIAL ASPECT OF THE FOREARM

Sites of cowhage application are indicated by stippling. The zones were surrounded by a narrow band, as indicated, of hyperalgesia.

insect bites, intracutaneous histamine puncture and intracutaneous foreign protein.

Pin prick applied not only locally but at considerable distance from the zone of itching abolished itching. With three subjects it was found that, if the cowhage was applied to the back, pin prick near the sternum in the same dermatome as the cowhage (Figure 2) was fully effective in eliminating itch.

In two subjects an attempt to delimit exactly the area within which pin prick abolished itching on the forearm was undertaken. Cowhage was applied at approximately the mid portion of the medial aspect of the forearm. After application of the cowhage the size of the area within which this effect could be demonstrated gradually increased and reached the eventual limit in about one hour following the beginning of itch. At this time the area measured approximately 5 × 15 cm. Figure 3 illustrates the area as mapped on the forearm of one subject on whom cowhage was applied as shown. It was essentially the same for the second subject.

After the mapping had been completed, cowhage was applied at the distal end of the area. The effect of pin prick was again tested, and it was found that the area within which it abolished itching was approximately the same as in the first instance, extending only 1–2 cm. further distally. Around this zone was a band of hyperalgesia to pin prick about 4 mm. wide.

A similar experiment was carried out on the legs of three subjects (Figure 4). The cowhage was applied just below the knee on the outer aspect of the left leg. Again there was an area within which the pin prick was effective in abolishing itch. After 60 minutes the area extended 7 cm. proximally and 24 cm. distally to the itching spot. On the leg, also, there was only slight difference in the area of effectiveness of the pin prick if the cowhage was shifted to another point within this area. A narrow band of hyperalgesia also surrounded this zone on the leg.

### *Comment*

Scratching is known to relieve itch. It has been held (1) that the relief so obtained resulted from replacement of the itch with frank pain. In the above observations the painful stimulus of the pin prick was not only brief but also far removed, as much as 24 cm. on the leg or back from the itching area, yet the itching sensation could thus be obliterated. Furthermore, the itch did not return until some time after the pain from the prick had subsided.

These observations seem most readily explicable mainly in terms of central rather than peripheral processes, as will be discussed below. The areas mapped on the forearm fall within the limit of the first thoracic dermatome; those on the leg correspond closely to L–4 or L–5 as determined by Förster (5).

### *Experiment 5. Demonstration that a zone of secondary hyperalgesia is "anti-pruritic"*

A superficial branch of a cutaneous nerve on the volar surface of the forearm was located by means of

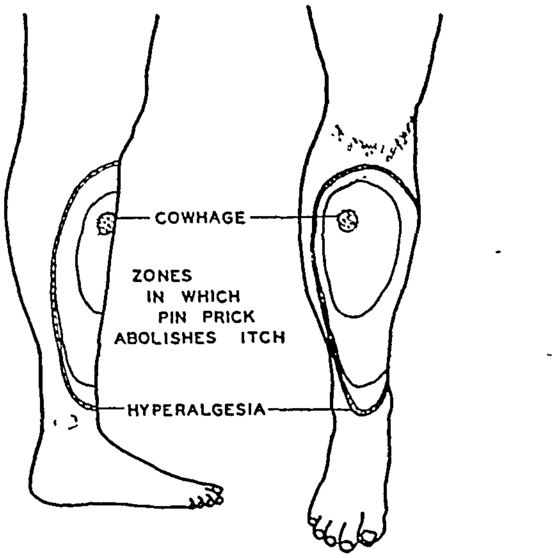

FIG. 4. ZONE ON THE LEG IN WHICH PAINFUL PIN PRICK ABOLISHED ITCHING INDUCED BY COWHAGE

The inner line indicates the extent of the zone after 15 minutes of itching; the outer border is its extent at the end of an hour. Sites of cowhage application are indicated by stippling. The zone was surrounded by a narrow band of hyperalgesia.

faradic current, and then was painfully stimulated for two minutes. Following this procedure there was established within 20 minutes in the surrounding skin a zone of "secondary hyperalgesia." In this zone of hyperalgesic skin the pain threshold as ascertained by the thermal radiation method was not lowered (10) but stimuli at threshold or above were perceived as more painful and longer lasting than stimuli of equal intensity in the surrounding skin.

Cowhage was applied in such an area of secondary hyperalgesia in four subjects in 11 observations. It evoked either occasional bursts of pain or no sensation at all, but not itching.

The secondary hyperalgesia was abolished in two subjects by infiltrating procaine at the site of injury by faradization and by vigorous pin pricks in a zone within 1–2 cm. around the point of faradization. Cowhage was again applied and itching now occurred as it did in control areas on the other arm.

In 12 other experiments on two subjects, areas of secondary hyperalgesia were induced by painfully pinching with forceps a small fold of skin on the volar surface of the forearm. Approximately 10 minutes after the pinch, when the secondary hyperalgesia was well developed, cowhage when applied failed to elicit itching. When the cowhage was pressed into the skin, a maneuver which intensifies itching in control non-hyperalgesic skin, pricking pain was experienced, but itch did not ensue.

### *Comment*

Lewis, Grant and Marvin (4) observed that faradic stimulation of a cutaneous nerve was followed by a state in adjacent skin in which itching did not occur after intracutaneous histamine puncture. Bickford (6) called this state "anti-pruritic" and found that it also occurred after other forms of noxious stimulation, such as a sharp blow, a gnat bite, burn or freeze. These authors, however, did not investigate the relation of hyperalgesia to the anti-pruritic state.

The observations on the effect of pin prick in abolishing itch, and on the failure of itching to occur in areas of secondary hyperalgesia, seem to indicate the existence of events of a special sort in the central nervous system. Similarity of the size and shape of the affected areas to the dermatome suggests that the spinal cord is the site of such processes. Bickford also concluded that the "anti-pruritic" state must depend on some spinal cord mechanism.

## *Discussion*

It has been held (1) that itching is a "protopathic," as opposed to an "epicritic" sensation. In these terms, the "first" itch might be considered epicritic, since it is readily localized, the second as protopathic since it is more diffuse. It is not clear, however, that any useful purpose is served by retaining this terminology.

The use of cowhage as a stimulus has been criticized on the grounds that the itching which results is impure (6). Histamine has therefore often been employed, but it was found that the itching which resulted was not nearly so intense as that produced by cowhage, and seemed to be chiefly of the "second" type. The "impurity" of the cowhage-provoked itching may well be simply the result of the simultaneous presence of both types of itch.

The objection might be raised that one of the two kinds of itch described above was not really itch, but was, instead, pain. The ultimate appeal in such a case must be to the introspection of the person experiencing the sensation, and the subjects in this investigation agreed that there were two kindred but distinguishable sensations which were both felt as "itch." They had in common their association with an urge to scratch the involved skin. Brack (18) also referred to two qualitatively different itch experiences.

It seems likely, in view of all the available data, that stimuli which produce itching initiate impulses which, after traversing the peripheral pain nerves, pass up the spinothalamic tracts of the cord. Since painful stimuli in general can be made to produce itching by lowering their intensity, it is reasonable to suppose that the difference between a painful and an itch-producing stimulus is one of intensity. The reduction in the pain threshold at times when itching is being experienced is further evidence in support of this view. The probable reason for the lowered threshold is that the peripheral pain endings are already partially activated by the cowhage, so that the radiant energy which must be applied to give pain is less than that necessary if they have not been stimulated at all.

Potelunas, Meixner and Hardy (19) reported that there was no consistent difference in the cutaneous pain thresholds of patients with itching dermatoses as opposed to healthy individuals. As they pointed out, however, structural changes such as thickening had often occurred in the diseased skin, with consequent alterations in pain sensitivity. All of the observations in the present investigations have been made on skin of subjects

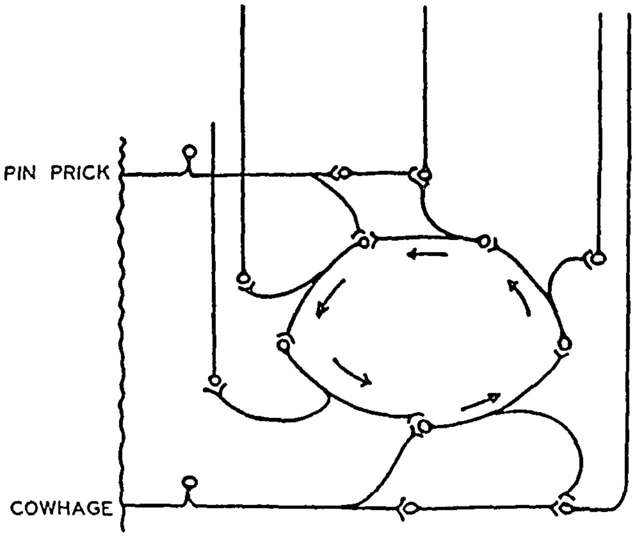

FIG. 5. SUGGESTED ARRANGEMENT OF CIRCUITS IN INTERNUNCIAL NEURONS RESPONSIBLE FOR ITCHING

Painful pin prick presumably breaks up the circuits.

without skin disorders, and without alteration of any sort except by the test procedures themselves. It must be emphasized that the threshold was found to be lowered only when the skin was itching.

One thesis which will explain all the findings is that the perception of itching depends on the presence in the cord of a circuit of internuncial neurons, analogous to those described by Lorente de No (20), around which impulses are constantly travelling. According to the hypothesis the establishment of such a circuit is the result of the low discharge frequency in a peripheral nerve which is weakly stimulated. As the circuit is traversed, discharge to spinothalamic neurons occurs, and hence impulses in the spinothalamic tract are sent upward to the brain in an orderly pattern (Figure 5). We suppose that the presence of this pattern of discharge in the brain is a necessary condition for the perception of itching. It is of interest that recently one subject following frontal lobotomy had an unaltered appreciation of itch, tickle and "itchy skin."

Such a circuit of excitation in an internuncial system of neurons would, of course, require a rather delicate adjustment of impulse frequencies and refractory periods. A pin prick in the dermatome in which itch is arising would, it is assumed, bring about a diffuse discharge in the corresponding cord segment, ramifying along many of the internuncial neurons involved in the itch circuit. The orderly pattern would therefore be temporarily destroyed, and itching halted. It would presumably require an appreciable time for it to be re-established, a time represented by the interval after pin pricking before itching is again perceived.

Hardy, Wolff and Goodell (10) have concluded that secondary hyperalgesia in the skin adjacent to a source of noxious impulses, results from the presence of an augmented central excitatory state in internuncial neurons. Such a state is indicated by the stipplings in Figure 6. It presumably acts to facilitate the passage of impulses at synapses. When cowhage is applied to skin in which such hyperalgesia is present, it may be supposed that the resulting impulses cannot set up the usual orderly circuits in the cord which result in itch sensation. The occasional burst of impulses elicited by the cowhage which is intense enough to cross the pain threshold of the pathway involved brings about a widespread discharge of the excitatory state and pain alone is experienced.

Such an explanation may outrun the data currently available about events in the human spinal cord, but on the other hand it does not do violence to any of the known facts of neural activity. The "all-or-none" law of impulse size in single nerve fibers does not come into question in this connection since differences in stimulus intensity, experienced as graded intensities and qualities of sensation, are generally considered to be reflected in differences in impulse frequencies in peripheral nerve fibers.

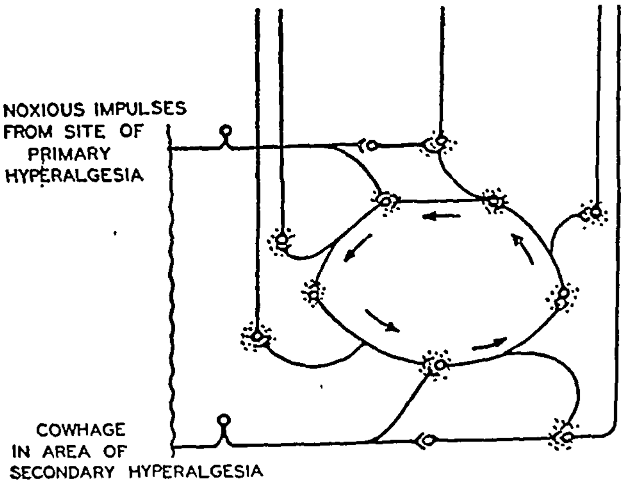
NOXIOUS IMPULSES FROM SITE OF PRIMARY HYPERALGESIA
COWHAGE IN AREA OF SECONDARY HYPERALGESIA

FIG. 6. INHIBITION OF ITCHING IN AREA OF SECONDARY HYPERALGESIA

The stippling represents the excitatory state of internuncial synapses which is responsible for the hyperalgesia and which prevents the formation of circuits necessary for itching.

## ITCHY SKIN

Lewis, Grant and Marvin (4) noted that spontaneous itching is an accompaniment of nearly all forms of skin damage, provided that the damage is relatively slight in amount. Bickford (6) further noted that itchy skin surrounds such skin damage. He described "itchy" skin as that which gives rise to an itching sensation when the skin is stroked with the finger, or a blunt object. He extensively investigated and described the phenomenon of "itchy skin" associated with the itch induced by intracutaneous histamine puncture and noted its close relationship to tickle. Goldscheider (12) described the occurrence of itching and "itchy skin" in the palm of the hand accompanying pinching of the web between two fingers. In the following investigations various modifications of both Goldscheider's and Bickford's experiments have been made in an attempt to illuminate further the mechanism of this phenomenon.

### *Experiment 6. Demonstration of "itchy skin" and hypoalgesia adjacent to a focus of itching*

Cowhage was applied to a small (1 cm. in diameter) area of skin on the volar surface of the forearm in various locations. It was found by trial that when it was placed on the wrist near the hand, or somewhat toward the medial aspect of the arm, the phenomenon of "itchy skin" could best be elicited. When the itch had become intense, usually within 30 to 60 seconds after application of the cowhage, stroking of the adjacent skin with the tip of a finger frequently induced an itchy sensation and more frequently intensified the primary itch. This zone extended approximately 2–3 cm. proximally, 1–3 cm. distally and 1–2 cm. laterally. Indeed, when the primary itch had entirely subsided, stroking the surrounding skin often revived it. Pin pricks in this surrounding "itchy skin" zone were dulled, but there was a narrow zone of hyperalgesia entirely surrounding it, where pin prick was experienced as sharper than in control skin. With the passage of time (30 to 60 minutes) after a long-lasting and intense itch, hyperalgesia was noted to have displaced the hypoalgesia and to extend 5 to 10 cm. proximally and 3 to 6 cm. distally. Such hyperalgesia after an intense itch sometimes persisted for several hours.

### *Experiment 7. Demonstration that the skin about a site of noxious stimulation is "itchy" until the development of secondary hyperalgesia*

In 12 experiments on three subjects a small fold of skin on the volar surface of the forearm was painfully pinched by forceps for two to eight minutes. The following description of the observations in an experiment in one subject is characteristic of all of this series (Figure 7). Pin pricks in a 1 cm. wide zone surrounding the pinch during the first 15 seconds intensified the pain of the pinch and there was *hyper*algesia immediately surrounding the pinch. Within the first minute a wider zone of *hypo*algesia to pin prick had developed and extended for 3 cm. proximal and distal to the pinch. In this zone stroking with the finger induced an itchy sensation which was indistinguishable from intense tickle, and itch was readily induced by cowhage. Surrounding this zone there was a narrow band of secondary hyperalgesia in which itch could not be induced either by stroking or by cowhage. In four minutes the hypoalgesic zone extended 3 cm. distally and 5 cm. proximally from the pinch.

It was repeatedly noted that pin pricks within the hypoalgesic zone, although they felt less sharp, accentuated the pain from the pinch. Furthermore, the pain elicited by the forceps gradually diminished. In the fifth, sixth, and seventh minutes when pain was no longer experienced, even rubbing the adjacent area with a finger or a blunt tongue depressor evoked a sensation of itching in the skin as well as at the point of the pinch.

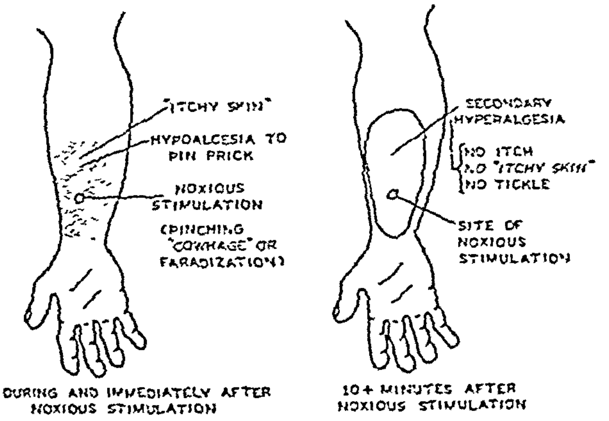
"ITCHY SKIN"
HYPOALCESIA TO PIN PRICK
NOXIOUS STIMULATION
(PINCHING "COWHAGE" OR FARADIZATION)
DURING AND IMMEDIATELY AFTER NOXIOUS STIMULATION
SECONDARY HYPERALGESIA
NO ITCH
NO "ITCHY SKIN"
NO TICKLE
SITE OF NOXIOUS STIMULATION
10+ MINUTES AFTER NOXIOUS STIMULATION

FIG. 7. SENSORY PHENOMENA IN THE SKIN ASSOCIATED WITH NOXIOUS STIMULATION

The pinch was maintained for seven minutes, when the "itchy" area was found to extend 4 cm. distally and 10 cm. proximally from the injury, still surrounded by a narrow band of hyperalgesia. Immediately after removal of the forceps, the hypoalgesic area diminished, its outer borders receding. The hypoalgesia was thus gradually replaced by hyperalgesia. In five to 10 minutes after removal of the forceps, the *hyper*algesia completely filled the zone which had been *hypo*algesic and "itchy." Cowhage applied to this hyperalgesic skin induced no itch, nor was it "itchy" in response to stroking.

The observations in Experiments 6 and 7 of hypoalgesia to pin prick in skin surrounding sites stimulated either by cowhage or by pinching prompted further study of this phenomenon. The experiments of Lewis, and of Hardy, Wolff and Goodell, in which they evoked secondary hyperalgesia by faradic stimulation, were repeated as follows:

*Experiment 8. Demonstration of hypoalgesia in skin adjacent to a focus of faradic stimulation*

In three subjects a branch of a cutaneous sensory nerve on the volar surface on the forearm was painfully stimulated with faradic current for four minutes. The adjacent skin was tested at the end of one minute and pin prick was found to be dulled. In the fourth minute just before the stimulation ended the pin was appreciated more as pressure than as pain. However, each prick markedly increased the pain from the faradic stimulation. Immediately after the end of the stimulation the hypoalgesic zone extended 4 cm. proximally, 6 cm. distally and 1 cm. laterally from the site of injury. Within 10 minutes no hypoalgesia could be detected. There was now a zone of marked hyperalgesia which extended 8 cm. proximally from the site of faradic stimulation, and 6 cm. distally. No itch or tickle could be elicited by drawing a thread across this area.

### *Comment*

It has been observed when an individual is experiencing one pain that the threshold for pain elsewhere is raised (17). Thus, one possible explanation of the hypoalgesia which was noted in the vicinity of the injured skin in Experiments 7 and 8 might be such an elevation of pain threshold. However, this cannot be the only explanation, because the hypoalgesia was limited in extent and persisted despite decreasing pain intensity as adaptation to the forceps pinch occurred, and for a few minutes after the removal of the forceps. This phenomenon might easily escape attention unless the adjacent skin area were tested during the period of noxious stimulation.

The observations of the last three experiments correspond to those of Experiment 5, in that skin in which secondary hyperalgesia existed was not "itchy" to stroking nor could itching be induced in it by cowhage. Also tickle was not elicited in the zone of secondary hyperalgesia. In short, although itchiness and secondary hyperalgesia may both be associated with skin damage, they do not occur in the same area at the same time.

*Experiment 9. Demonstration of the occurrence of both "itchy skin" and itching associated with skin injury*

Goldscheider's experiment was repeated in a series of 20 experiments on three subjects. The web between two fingers was tightly pinched with fine forceps. Within half a minute not only could "itchy skin" be demonstrated on stroking of the palm and the back of the hand, but also itch occurred in the palm of the hand spontaneously. Again the "itchy skin" was hypoalgesic to pin prick and was surrounded by a band of hyperalgesia which was *not* "itchy." There was also a small area of hyperalgesia a few millimeters in diameter at the site of the pinch, presumably primary hyperalgesia.

Similarly, pinching a web between the toes for less than two minutes gave rise to spontaneous itching and "itchy skin" in the sole of the foot, especially on the underside of the great toe and the ball of the foot. In one of the subjects, the itching induced by such slight injury between the toes continued for more than an hour.

In one subject itching in and around an insect bite on the medial aspect of the left thigh, about eight inches above the knee, persisted for three days. On the third day a zone of "itchy skin" was found to extend for 5 to 6 cm. around the reddened bite, which was itself spontaneously itching, and hyperalgesic to pin prick. In the zone of "itchy skin" pin prick was dulled, but the area was surrounded by a clearly perceptible narrow band of hyperalgesia. A few pin pricks within this area abolished the itching from the bite for 30 to 60 seconds.

Similar observations were made in a second subject who had "itchy skin" on the top of the left foot associated with a slight abrasion of the skin.

*Experiment 10. Demonstration of the spread of itching from a primary focus of itching*

In a series of four experiments on two subjects cowhage was placed on an area of skin approximately 1 cm. in diameter on the volar surface of the wrist at the junction between the hand and the arm. Accompanying the intense itch elicited on this site not only was the itchiness in response to stroking of adjacent skin marked, but spontaneous itching which spread into the thenar and hypothenar eminences and into the palm of the hand occurred. Here, again, the area of itchy skin was surrounded by a narrow zone of hyperalgesia which was not "itchy."

### *Comment*

Goldscheider reported that painfully pinching the skin led to the development of hyperalgesia in the surrounding area. He apparently did not notice that the more remote border of hyperalgesia was only a narrow zone and in fact surrounded an area of *hypo*algesia to pin prick. It is only after the passage of several minutes following a pinch of four to seven minutes duration that the hyperalgesia completely displaces the hypoalgesia. Goldscheider inferred that the "itchy skin" was related to hyperalgesia, but from the experiments reported here, it appears that only the skin which is *hypo*algesic to pin prick is "itchy" in response to stroking. These findings are in agreement with the observations that itch is not induced by cowhage in a zone of secondary *hyper*algesia. This is evidence of fundamental similarities between "spontaneous itching" which follows the application of cowhage or histamine, and that which can be elicited by stroking in skin adjacent to a source of noxious impulses.

*Experiment 11. Demonstration of the relation of "itchy skin" and pain, and of the occurrence of "itchy skin" in the absence of touch sensation*

In three subjects a blood pressure cuff was wrapped around the upper arm, inflated to 200 mm. Hg, and kept at this pressure for the duration of the experiment. At the end of eight to 15 minutes when pin prick was slightly dulled in the palm of the hand, drawing the end of a tongue depressor across the palmar skin elicited an intense burning itch, and stroking with a finger also elicited tickle or itch. At the end of 20 minutes in all three subjects the sensation of touch in response to laying the flat side of the tongue depressor on the wrist was gone. Itch powder (cowhage) was then applied and within two minutes elicited a burning itch. Stroking the skin both proximal and distal to the cowhage, was felt as "itchy" and also intensified the itch from the cowhage. The zone in which "itchy" skin could be detected was well demarcated for a distance of 8–10 cm. proximal to the itch spot, and throughout the palm to the tips of the fingers. In the distal zone of "itchy skin" no touch sensation could be detected.

### *Comment*

Bickford reported that the perception of "itchy skin" failed at a stage of asphyxia at which both touch and pain sensibility were still present, and was therefore forced to postulate that special peripheral nerves transmitted the sensation. The observations described above, however, are evidence that "itchy skin" can occur in the absence of touch as long as pain is preserved.

They indicated that both itch and "itchy skin" occur independently of touch sensation, and as long as some fibers are still conducting pain impulses. Perhaps Bickford failed to make this observation because he used intracutaneous histamine puncture to produce spontaneous itching and "itchy skin" and in our experience the latter was less intense and more difficult to detect when histamine was used to evoke itch.

### *Discussion*

The "itchy skin" phenomenon is indistinguishable subjectively from tickle (see next section). The sensation evoked by light stroking of otherwise unstimulated skin is called "tickle," that evoked by light stroking of skin in the neighborhood of a source of itching has been called "itchy," but they are otherwise alike, and both provoke a desire to scratch. "Itchy skin" is like itch and tickle in that it could not be elicited in skin manifesting secondary hyperalgesia. Bickford observed that if it was impossible to elicit "itchy skin" as a result of nervous system disease or of experimental procedures, it was also impossible to elicit tickle. He concluded that the development of "itchy skin" depended on axon-reflexes similar to those responsible for the development of the flare around sites of skin injury. Whether or not this is correct, the "itchy" sensation elicited in the area presumably results from a kind of activity in internuncial neurons similar to that which is responsible for the primary itching at the site of cowhage application.

## TICKLE

When a stiff nylon thread is drawn across the skin a peculiar esthetic experience usually ensues, which long outlasts the period of stimulation. This is best demonstrated on the margin of the upper lip. The sensation has two distinct components. The first of these resembles itch and has in common with it an associated urge to rub or scratch the skin. The second consists of an awareness of movement of a light object touching the skin. In some parts of the body, *e.g.*, the finger pads and the extensor surface of the elbow, only the second component is prominent, whereas at the lip margin the itching component is conspicuous. It is for the itching component of the sensation evolved by a moving stimulus that the term "tickle" has been reserved in this paper.

In the following experiments the relation of tickle, itch, "itchy skin" and pain were further investigated.

*Experiment 12. Demonstration that tickle and itch differ only in that tickle has the additional element of movement*

A fine nylon thread was touched to the skin of the forehead in one spot. Ten subjects so tested reported that the sensation elicited was itch, and was accompanied by the urge to rub or scratch. When the thread was lightly *drawn across* the skin, some of the subjects changed their report of the sensation to tickle, but stated that the only change in the quality of the experience was the additional perception of movement of the thread, although the desire to rub or scratch was intensified.

In six subjects, the difference between touch and tickle or itch was clearly demonstrated by touching first the pad of one finger and then the forehead. A light touch by the finger of the experimenter on the finger pad of the subject elicited only touch, but on the forehead, itch or tickle were elicited, with an invariable accompaniment of a desire to rub or scratch the stimulated skin.

*Comment*

These observations make clear that the conception of tickle in everyday experience conforms exactly to the definition given above, *i.e.*, tickle is the itching component of the sensation evoked by a light moving stimulus on the skin.

*Experiment 13. Demonstration that tickle is obliterated by stimulation of pain endings*

The forehead was rubbed briskly so that a slight after-pain was experienced, and again tested by lightly drawing the nylon thread across the skin. Now the sensation elicited was purely that of touch without the unpleasant component arousing the urge to rub or scratch. Similarly, immediately after the forehead was pricked vigorously with a pin, for at least as long as the faint after-pain of the pricking persisted the sensation elicited by the thread was again only touch.

*Comment*

Although drawing a wisp of cotton across the skin in most skin areas is used as a bedside testing procedure to indicate perception of light touch, it is also used on the upper lip, a very "ticklish" area, to test for the integrity of fiber systems conveying noxious impulses in the skin supplied by the fifth cranial nerve.

It is apparent that the drawing of a thread or wisp of cotton across the skin may under varying circumstances give rise to qualitatively distinct sensations. As stated above, it has seemed profitable to reserve the word "tickle" for that part of the sensation thus elicited which resembles itch and arouses the desire to rub or scratch. The effect of painful stimuli in abolishing or preventing tickle is apparently analogous to the phenomenon of abolishing itch by pin prick.

*Experiment 14. Demonstration of the absence of tickle in a zone of secondary hyperalgesia*

In two subjects zones of secondary hyperalgesia were defined on the volar surfaces of the forearms after faradic stimulation of a superficial cutaneous sensory nerve. When the hyperalgesia was fully developed a nylon thread drawn lightly across the area elicited a sensation of touch; when the thread was applied with slightly greater force, touch commingled with pain was elicited, but the sensation was definitely not tickle. The same thread elicited tickle in control areas on the opposite arm, and in areas of skin on the same arm outside of the area of secondary hyperalgesia. In these two subjects the secondary hyperalgesia was abolished by pin prick within its borders. Immediately thereafter, tickle could again be elicited.

*Comment*

It appears that tickle, like itch, is inhibited by the presence of secondary hyperalgesia of the skin; that a stimulus clearly eliciting tickle in control skin, elicits in hyperalgesic skin either a sensation of touch without the special quality of tickle or itch, or of touch commingled with pain. Areas of secondary hyperalgesia have thus been demonstrated to be "anti-ticklish" as well as "anti-pruritic."

*Experiment 15. Demonstration of the occurrence of tickle in the absence of touch sensation*

In three subjects a blood pressure cuff was inflated about the upper arm at a pressure of 200 mm. Hg. About 20 minutes later the light touch of a flat tongue depressor could not be perceived on the back and side of the forefinger. At this time, however, a stiff nylon thread drawn across this area elicited a distinct tickle which merged into an itching sensation

*Comment*

It has been previously noted that during asphyxia of an arm touch sensation disappears at a time when pain can still be elicited. This experiment demonstrates that tickle, like itch, is independent of touch, and is indeed also probably carried over the afferent fibers which mediate pain.

## *Discussion*

No general agreement on the relation of the sensation of tickle to touch, pain and itch has previously been reached. Discussion has focussed on whether tickle was essentially different from itch, and whether the mediation of tickle sensation was to be assigned to touch or to pain receptors.

With regard to the first question, Murray (21) reported that her subjects found that itch differed from tickle only in being "more persistent, painful and intolerable." Török (22), on the other hand, asked patients with pruritus to compare their spontaneous itching with the sensation elicited by drawing a wisp of cotton across the skin. From these comparisons he concluded that itch and tickle were different sensations which could be distinguished by his subject. It should be noted, however, that motion adds an element to the sensation which makes it different from itching, and probably accounts for the patients' statements that they could distinguish between the two. It is also important that under certain circumstances, as discussed above, the same moving stimulus provokes a sensation which induces no desire to scratch, consists entirely of touch, and is not ordinarily called tickle by the subject experiencing it. It is conceivable that difficulties of communication with patients might lead to a report of "tickle" for this sensation also.

Thöle (11) also separated itch and tickle as regards the structures involved, concluding that tickle was related to stimulation of touch receptors. He failed to make the observation that tickle can be elicited in areas of skin in which touch is no longer present, nor did he differentiate between true tickle and a sensation of moving touch in areas where pain could not be elicited.

Bishop (23, 24) suggested that tickle sensation was a function of touch rather than pain receptors. He found that weak and rapid electrical stimulation of touch endings failed completely to elicit tickle but this he attributed to the lack of movement of the stimulus. He argued that tickle can be elicited by a contact with the skin so light that it is clear that the receptors concerned must have a very low threshold, and that therefore participation of pain receptors is out of the question. In his own observations on cutaneous sensation, however, he found that in most areas of skin, except notably on the balls of the finger, prick has a *lower* threshold than touch. Furthermore, in reviewing (25) von Frey's observations, Bishop calls attention to the fact that in using mechanical stimulators of small diameter, thresholds for touch and pain closely approximate each other. Likewise, Hardy, Wolff, and Goodell (10), testing for touch threshold with von Frey hairs in hyperalgesic and control areas of skin, found that the thresholds for touch and for pain were not grossly different. Bishop's second piece of evidence linking tickle with touch was that tickle, like touch, "adapts" to continued stimulation (24). However, continuous noxious stimulation also ceases to produce pain, as can be readily demonstrated by holding a pin point steadily against the skin for several seconds, or by pinching a fold of skin with forceps.

Bickford observed that tickle, "itchy skin," itch, and pain were all absent in patients with anterolateral tract lesions, even though touch sensation remained intact.

Pritchard (26) considered that tickle, itch and pain all lay on the same continuum of sensation, and that tickle, like itch, was a variety of pain. He nevertheless concluded that tickle and pain involved different peripheral pathways. He failed to present evidence that tickle could be elicited in the complete absence of pain. On the other hand, Sarnoff and Arrowood (27) reported that tickle sensation elicited "by scratching the soles of the feet" persisted in some of their patients in the absence of pain from pin prick during spinal block with procaine. However, the intensity of their scratch stimulus was not described, and it is not clear that it elicited true tickle, especially since there was no description of the qualities of the sensation experienced. It is also possible that their observations may be related to Thöle's findings (11).

Zotterman (28) on the basis of his studies of axone potentials in cat nerves concluded that tickle and itching sensations are mediated by the fibers which are responsible for the pain elicited by pin prick. He also observed that tickle could not be elicited in patients with analgesia of the face following trigeminal tractotomy, although touch sensibility was intact.

On the basis of the observations reported here it is inferred that the sensation called "tickle" is mediated by the same neural fibers as are involved in itch and pain. This conclusion is based on the experiments involving differential anesthetization, for tickle could be elicited when touch sensitivity was absent and pain sensitivity retained, thus exactly paralleling cowhage-induced itching in like circumstances. Furthermore, tickle is inhibited in an area of secondary hyperalgesia, as is itch; and it is obliterated in skin to which a painful pricking or rubbing has been applied, just as itch is obliterated by pricking in adjacent areas of skin. It is concluded, therefore, that the pathways concerned in the perception of tickle are those for pain, and that touch mechanisms do not participate, except to add something which is essentially extraneous.

## SUMMARY AND CONCLUSIONS

1. The sensation of itch has two subjectively distinguishable components, one pricking and the other burning. These correspond to the two kinds of cutaneous pain, and are mediated respectively by the two types of nerve fibers involved in the transmission of pain from the skin. Touch receptors and fibers are not involved.

2. Cutaneous tickle and the sensation elicited in "itchy skin" do not differ qualitatively from itch, except by the addition of an awareness of movement, and are mediated by the same neural structures, *i.e.*, those which transmit pain. Touch receptors, when functioning, probably add an essentially extraneous component to tickle and to the sensation elicited in "itchy skin," but both phenomena occur in the absence of touch.

3. When itching is present, the pain threshold at the site is lower than it is in the same skin during itch-free intervals.

4. Itch occurs when pain receptors are weakly stimulated.

5. Tickle, itchy skin and itch are abolished by pin pricks in adjacent skin. In the case of itch, this abolition is possible if the skin is pricked anywhere in the dermatome which contains the site of itching.

6. Tickle, "itchy skin," and itch do not occur in areas of secondary hyperalgesia. They do occur in skin surrounding sites of noxious stimulation when the area is hypoalgesic to pin prick.

7. It is suggested that the sensation of itch results from the presence in the spinal cord of impulses traveling in circuits of internuncial neurons, with a consequent patterned discharge up the spinothalamic tracts. Such circuits are presumably established when peripheral pain nerves discharge into the cord at a low frequency. When the circuits are broken up—by strong stimulation in the same dermatome, for instance—itching ceases.

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# THE EFFECT OF ORAL ADMINISTRATION OF CALCIUM FRUCTOSE DIPHOSPHATE ON THE SERUM ORGANIC PHOSPHATE, INORGANIC PHOSPHATE, CALCIUM, PROTEIN, AND CITRIC ACID LEVELS[1]

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(Submitted for publication June 22, 1950; accepted, October 17, 1950)

It has been clearly demonstrated that the rate of glucose absorption is independent of the concentration of the glucose solution introduced into the intestine (1–4). This has been interpreted as indicating that glucose absorption is an enzymatic process in which phosphorylation at the wall of the intestine precedes the absorption of glucose (5–7). This mechanism has been thoroughly tested and evidence in its favor is convincing (8–10). It appeared to us, therefore, that hexosephosphates introduced into the intestinal tract might be absorbed directly, without splitting of the phosphate linkage with the exception of that material which would be hydrolyzed by stomach acids and destroyed by the intestinal flora. It was therefore decided to determine the manner in which a hexosephosphate given orally to adults is absorbed. The adults studied were patients taken from our wards who showed no evidence of impaired intestinal absorption.

The administration of large amounts of inorganic phosphate is not an innocuous procedure in that one runs the risk of reducing the calcium ion concentration to a low level with resultant tetany. It was therefore decided, in this preliminary study, to administer calcium simultaneously. This could conveniently be done by administering the hexosephosphate in the form of its calcium salt. Calcium fructose diphosphate is readily available and was, therefore, the substance chosen.

The calcium fructose diphosphate was prepared by the Ernst-Bischoff Company,[3] in the form of a fine powder, and also in pills containing 0.5 gram of this material. If we assume that one molecule of this material contains two atoms of calcium and two phosphate groups, then the empirical formula is $C_6H_{12}O_{12}P_2Ca_2$ and the molecular weight, 418. Theoretically, 14.8 per cent phosphorus and 19.1 per cent of calcium should be present in the molecule. On analyzing the product, 13.6 per cent of phosphorus was obtained and 17.2 per cent of calcium.[3]

Calcium fructose diphosphate is apparently readily hydrolyzed. It was observed that preparations which had been kept in stoppered bottles, but which had been opened frequently over a nine months' period, lost a high percentage of their organic phosphate content, the phosphate in the material becoming inorganic. The material used, therefore, was tested for its inorganic phosphate content. If the material contained more than 2 per cent of its phosphate in the inorganic form, it was discarded. Calcium fructose diphosphate is more soluble than calcium phosphate, and a preliminary test may be made by shaking up 20 mg. in 25 ml. of water. The material should dissolve readily with no residue.

The criteria of whether the hexosephosphate has reached the blood stream were determined by the organic phosphate content of the serum after the proteins had been removed. Inorganic phosphate was also measured. Calcium and two substances which normally form complexes with calcium, namely, citric acid and protein, were also estimated. Calcium was done by the method of Sobel and Sobel (11). Inorganic phosphorus was done by a modified method of Fiske and Subbarow (12, p. 579). Total protein was done by a modified biuret method (13). Citric acid was done by the method of Natelson, Pincus, and Lugovoy (14). Determination of total non-protein phosphorus was done by digestion of the trichloracetic acid filtrate of cell free serum (12, p. 581). When 50 μg. of phosphorus as calcium fructose diphosphate or as calcium glyceryl monophosphate were added per ml. of serum, 94 to 102 per cent of the phosphorus added was recovered by this method.

As determined by recoveries of added amounts of various constituents to serum, citric acid was determined with an accuracy of 5 per cent, inorganic phosphorus with an accuracy of 3 per cent and calcium with an accuracy of 3 per cent. Per ml. of serum, 20 μg. of citric acid, 40 μg. of inorganic phosphorus, and 50 μg. of calcium were added for the recovery determinations. The accuracy of the protein method was estimated at approximately 3 per cent by comparing the procedure used with the Kjeldahl procedure on 50 samples, in the routine laboratory. All analyses were done in duplicate and the tables below show the results of the average of these duplicates.

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[1] Presented at the Federation Meeting of the American Societies for Experimental Biology at Atlantic City, April, 1950.

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[3] Calcium fructose diphosphate is manufactured by the Ernst Bischoff Co. of Ivorytown, Conn., under the trade name of Com-B-Phos. The material supplied us contained some water of crystallization. The company makes an anhydrous product which they claim is more stable.

The experiments were performed in the following manner: A fasting sample of blood was drawn and 6 grams (12 pills), of calcium fructose diphosphate were taken by mouth. Thereafter, samples of blood were taken at intervals of one hour for three hours.

TABLE I

*Change in serum levels after administration of 6 grams of calcium fructose diphosphate*

| Case* no. | Time | Non-protein phosphorus (*mg.* %) | | | Calcium | Citrate | Protein |
|---|---|---|---|---|---|---|---|
| | | Total | Inorganic | Organic | | | |
| | *hrs.* | | | | *mg.* % | *mg.* % | % |
| 1 | Fasting | 3.9 | 3.3 | 0.6 | 10.1 | 2.03 | 7.3 |
| | 1 | 5.8 | 3.2 | 2.6 | 10.3 | 1.91 | 7.5 |
| | 2 | 6.7 | 3.3 | 3.4 | 10.5 | 2.03 | 7.3 |
| | 3 | 6.1 | 3.5 | 2.6 | 10.4 | 2.00 | — |
| 2 | Fasting | 3.1 | 2.7 | 0.4 | 9.7 | 1.89 | 6.3 |
| | 1 | 5.6 | 2.9 | 2.7 | 10.3 | 2.20 | 6.4 |
| | 2 | 6.1 | 3.4 | 2.7 | 10.2 | 1.93 | 6.2 |
| | 3 | 4.7 | 3.0 | 1.7 | 10.3 | 2.03 | 6.5 |
| 3 | Fasting | 3.4 | 3.0 | 0.4 | 9.0 | 2.34 | 7.2 |
| | 1 | 4.4 | 3.7 | 0.7 | 9.3 | 2.56 | 7.3 |
| | 2 | 5.8 | 4.1 | 1.7 | 9.6 | 2.33 | 7.5 |
| | 3 | 6.7 | 3.8 | 2.9 | 9.6 | 2.28 | 7.3 |
| 4 | Fasting | 3.5 | 2.9 | 0.6 | 8.3 | 2.44 | 5.6 |
| | 1 | 4.4 | 3.1 | 1.3 | 8.5 | 2.44 | 5.7 |
| | 2 | 7.5 | 3.1 | 4.4 | 8.8 | 2.71 | 5.8 |
| | 3 | 5.3 | 3.1 | 2.2 | 8.3 | 2.49 | 5.6 |
| 5 | Fasting | 4.7 | 3.5 | 1.2 | 10.3 | 2.25 | 5.9 |
| | 1 | 5.8 | 3.7 | 2.1 | 10.5 | 2.70 | 5.9 |
| | 2 | 7.0 | 4.3 | 2.7 | 10.6 | 2.05 | 6.1 |
| | 3 | 5.8 | 4.3 | 1.5 | 10.6 | 2.20 | 6.1 |
| 6 | Fasting | 5.6 | 4.5 | 1.1 | 9.5 | 1.95 | 6.9 |
| | 1 | 7.0 | 5.0 | 2.0 | 10.3 | 2.40 | 6.9 |
| | 2 | 8.1 | 5.0 | 3.1 | 10.3 | 1.95 | 7.0 |
| | 3 | 7.0 | 5.0 | 2.0 | 10.3 | 1.95 | 7.2 |
| 7 | Fasting | 4.9 | 4.2 | 0.7 | 10.1 | 2.36 | 7.1 |
| | 1 | 5.7 | 4.4 | 1.3 | 11.0 | 2.42 | 7.3 |
| | 2 | 5.6 | 4.5 | 1.1 | 10.9 | 2.61 | 7.2 |
| | 3 | 5.3 | 4.6 | 0.7 | 10.8 | 2.29 | 7.3 |
| 8 | Fasting | 3.9 | 3.4 | 0.5 | 10.2 | 1.84 | 6.5 |
| | 1 | 5.0 | 3.6 | 1.4 | 10.9 | 2.06 | 6.6 |
| | 2 | 5.1 | 3.7 | 1.4 | 10.8 | 2.06 | 6.6 |
| | 3 | 4.6 | 3.5 | 0.9 | 10.8 | 2.03 | 6.5 |
| 9 | Fasting | 3.7 | 2.6 | 1.1 | 9.9 | 2.36 | 6.7 |
| | 1 | 4.2 | 2.8 | 1.4 | 10.2 | 2.51 | 6.7 |
| | 2 | 4.7 | 2.9 | 1.8 | 10.2 | 2.36 | 6.8 |
| | 3 | 5.2 | 3.0 | 2.2 | 10.4 | 2.30 | 6.9 |
| 10 | Fasting | 4.6 | 3.4 | 1.2 | 10.2 | 2.18 | 6.8 |
| | 1 | 5.0 | 3.4 | 1.6 | 10.2 | 2.30 | 6.7 |
| | 2 | 6.9 | 4.0 | 2.9 | 10.4 | 2.18 | 6.8 |
| | 3 | 6.5 | 3.9 | 2.6 | 10.5 | 2.14 | 6.8 |
| 11 | Fasting | 3.7 | 3.5 | 0.2 | 9.8 | 1.60 | 7.2 |
| | 1 | 4.2 | 3.8 | 0.4 | 9.8 | 1.70 | 7.6 |
| | 2 | 4.2 | 3.8 | 0.4 | 10.3 | 1.86 | 7.4 |
| | 3 | 4.4 | 4.1 | 0.3 | 9.4 | 1.43 | 7.4 |
| 12 | Fasting | 4.2 | 3.9 | 0.3 | 8.1 | 2.10 | 7.5 |
| | 1 | 5.0 | 4.5 | 0.5 | 8.5 | 2.31 | 7.7 |
| | 2 | 5.5 | 4.8 | 0.7 | 8.0 | 2.14 | 7.4 |
| | 3 | 5.3 | 4.8 | 0.5 | 8.3 | 1.90 | 7.4 |

* Hospital patients with apparently no impairment of intestinal function. Results are averages of duplicates.

Results are shown in 12 cases in Table I (cases 1 through 12). Summary of these results is shown in Tables II and III where the various determinations are averaged for cases 1 through 12. Table IV shows the results carried out with a calcium fructose diphosphate powder preparation which had been hydrolyzed by nine months' exposure to moisture in the powder and air. On analysis, approximately 80 per cent of the phosphorus was in inorganic form. These results serve as a "blank" for comparison of the absorption of a hexosephosphate with a phosphate preparation which contained the same quantity of calcium and phosphorus, but little organic phosphate.

## DISCUSSION

Examination of Table III shows a rise of approximately 15 per cent in inorganic phosphorus, and a rise of 173 per cent of the non-protein organic phosphorus, over the fasting level. That these results are statistically significant is apparent from Table I where increases are observed in every case without exception. The control table (Table IV) indicates that the total rise in organic phosphorus levels could not be caused by the ingestion of inorganic phosphate. In view of the small amount of organic phosphate normally present in cell-free serum, the 173 per cent rise is somewhat misleading. An estimation of the amount of organic phosphate absorbed is best obtained by examination of the average absolute rise in non-protein organic phosphate. This is listed as 1.40 mg. per 100 ml. in Table III. Assuming that 20 per cent of the body weight represents total extracellular fluid, the average adult would have 20 per cent × 75 Kg. = 15 liters of extracellular fluid. The total non-protein organic phosphorus increase would then be $1.4 \times 10 \times 15 \div 1000 = 0.210$ gram. Since the phosphorus represents only 13 per cent of the calcium fructose diphosphate

TABLE II

*Average values for the 12 cases studied*
*(Table I, cases 1–12)*

| | Calcium | | | Total protein | | | Citric acid | | |
|---|---|---|---|---|---|---|---|---|---|
| | Mg. % | Mg. % rise over fasting | % Rise over fasting | Mg. % | Mg. % rise over fasting | % Rise over fasting | Mg. % | Mg. % rise over fasting | % Rise over fasting |
| Fasting | 9.59 | — | — | 6.76 | — | — | 2.06 | — | — |
| 1 hr. | 9.90 | 0.31 | 3.2 | 6.80 | 0.04 | 0.6 | 2.28 | 0.22 | 10.7 |
| 2 hrs. | 10.01 | 0.42 | 4.4 | 6.80 | 0.04 | 0.6 | 2.19 | 0.13 | 6.8 |
| 3 hrs. | 9.95 | 0.36 | 3.8 | 6.73 | 0.03 | 0.4 | 2.10 | 0.04 | 1.9 |

used, this would imply that 1.6 grams of fructose diphosphate is present in the extracellular fluid at the time of the peak rise in nonprotein organic phosphorus as an average for the 12 adults. The one hour, two hour and three hour figures in Table III for organic phosphorus indicate that a continuous process of absorption and metabolism is going on. From this observation and the fact that some fructose diphosphate is probably hydrolyzed by the acid in the stomach, one might infer that a high percentage of the fructose diphosphate which reaches the intestine is rapidly absorbed. The speed of absorption is indicated by the fact that a rise in non-protein organic phosphorus can be demonstrated in one hour and that a peak is reached in two hours. This compares in speed with the absorption of glucose. A more accurate picture would have been obtained if enteric coated pills had been used.

It is of interest that at no time did the inorganic phosphorus levels rise to a point where a hazard of tetany might be encountered. At the peak, the inorganic phosphorus rise was less than one-third that of the rise in organic non-protein phosphorus. This rise in inorganic phosphorus is equivalent to 0.59 gram of calcium fructose diphosphate. Thus at the peak rise, more than one-third of the substance administered (6 grams) could be accounted for in the extracellular fluid (1.6 grams + 0.59 gram = 2.19 grams), calculated on the rise in total phosphorus.

The ratio of the phosphorus to calcium absorbed should have been 31/40 or approximately 3/4 if the calcium fructose diphosphate molecule had been absorbed directly. Actually the ratio was 1.91/0.42 (see Tables III and IV), or 9/2 approximately. Disproportion between the absorption of calcium and organic phosphorus may be interpreted on the basis that the molecule is not absorbed as a whole, but that in the intestinal tract calcium dissociates, and the fructose diphosphate is absorbed in greater amounts than calcium. This could only be demonstrated if the amount of calcium excreted in the stool were measured. In the present study stool analyses were not done.

The relatively slight increase in calcium concentration (Table II) suggested that a large portion of the calcium may be tightly bound in organic form. However, ashing made no significant difference (2 per cent increase) in the calcium content of the same serum at the peak organic phosphorus level. Increase in citrate levels is

TABLE III

*Average values for the 12 cases studied*
*(Table I, cases 1–12)*

| | Total non-protein phosphorus | | | Inorganic phosphorus | | | Organic non-protein phosphorus | | |
|---|---|---|---|---|---|---|---|---|---|
| | Mg. % | Mg. % rise over fasting | % Rise over fasting | Mg. % | Mg. % rise over fasting | % Rise over fasting | Mg. % | Mg. % rise over fasting | % Rise over fasting |
| Fasting | 4.19 | — | — | 3.40 | — | — | 0.79 | — | — |
| 1 hr. | 5.17 | 0.98 | 23 | 3.66 | 0.26 | 7.65 | 1.51 | 0.72 | 92 |
| 2 hrs. | 6.10 | 1.91 | 46 | 3.91 | 0.51 | 15.00 | 2.19 | 1.40 | 173 |
| 3 hrs. | 5.58 | 1.39 | 33 | 3.88 | 0.48 | 14.10 | 1.70 | 0.91 | 116 |

TABLE IV

*Change in serum levels after administration of 6 grams of hydrolyzed calcium fructose diphosphate*

| Case* no. | Time | Non-protein phosphorus (mg. %) | | | Calcium | Citrate | Protein |
|---|---|---|---|---|---|---|---|
| | | Total | Inorganic | Organic | | | |
| | *hrs.* | | | | *mg. %* | *mg. %* | *%* |
| 13 | Fasting | 4.2 | 3.9 | 0.3 | 9.0 | 1.90 | 6.0 |
| | 1 | 4.6 | 4.1 | 0.5 | 9.6 | 2.20 | 6.0 |
| | 2 | 4.6 | 4.1 | 0.5 | 9.5 | 2.10 | 6.2 |
| | 3 | 4.6 | 4.2 | 0.4 | 9.4 | 1.90 | 6.0 |
| 14 | Fasting | 4.4 | 3.8 | 0.6 | 10.0 | 2.20 | 7.4 |
| | 1 | 5.0 | 4.3 | 0.7 | 10.0 | 2.10 | 7.5 |
| | 2 | 5.3 | 4.5 | 0.8 | 10.3 | 2.33 | 7.2 |
| | 3 | 5.5 | 4.8 | 0.7 | 10.1 | 2.25 | 7.2 |
| 15 | Fasting | 3.5 | 3.0 | 0.5 | 10.0 | 2.20 | 7.3 |
| | 1 | 3.9 | 3.3 | 0.6 | 10.3 | 2.36 | 7.6 |
| | 2 | 4.3 | 3.5 | 0.8 | 10.5 | 2.33 | 7.6 |
| | 3 | 4.8 | 4.0 | 0.8 | 10.2 | 2.33 | 7.3 |
| 16 | Fasting | 8.2 | 7.6 | 0.6 | 8.0 | 2.10 | 5.0 |
| | 1 | 8.2 | 7.4 | 0.8 | 8.7 | 2.54 | 5.4 |
| | 2 | 8.1 | 7.4 | 0.7 | 8.7 | 2.15 | 5.0 |
| | 3 | 8.2 | 7.5 | 0.7 | 8.1 | 1.92 | 5.0 |
| 17 | Fasting | 6.2 | 5.5 | 0.7 | 7.7 | 1.92 | 4.7 |
| | 1 | 6.0 | 5.5 | 0.5 | 8.1 | 2.03 | 4.8 |
| | 2 | 6.0 | 5.4 | 0.6 | 7.6 | 2.06 | 4.7 |
| | 3 | 6.2 | 5.4 | 0.8 | 7.6 | 1.96 | 4.7 |
| Average | Fasting | 5.3 | 4.8 | 0.5 | 8.9 | 2.06 | 6.1 |
| | 1 | 5.4 | 4.9 | 0.5 | 9.3 | 2.25 | 6.2 |
| | 2 | 5.6 | 5.0 | 0.6 | 9.3 | 2.20 | 6.1 |
| | 3 | 5.9 | 5.2 | 0.7 | 9.1 | 2.06 | 6.0 |

* Hospital patients with apparently no impairment of intestinal function. Results are averages of duplicates.

probably associated with the rise in calcium levels (15). Protein changes were not considered significant.

Certain pathological conditions exist such as hyperparathyroidism (16), and low phosphorus rickets (17–19), where the phosphate levels of the blood cannot be maintained even when inorganic phosphate is administered. In lead poisoning, the administration of phosphorus has been suggested in order to alleviate symptoms by removal of lead from the plasma (20–22). This technique has been hampered by the danger associated with injections of inorganic phosphorus. The authors intend to investigate the possibility that hexosephosphates may find application in these cases.

The properties ascribed to calcium fructose diphosphate in this report are apparently not limited to fructose diphosphate since calcium glycerol phosphate, when tested in one case, produced similar results. It is the intention of the authors to extend this study to other phosphate esters such as glucose 6 phosphate, and glucose 1 phosphate.

## SUMMARY

1. The effect of 6 grams of calcium fructose diphosphate administered orally on the calcium, protein, citric acid, inorganic phosphorus and non-protein organic phosphorus levels of the serum was studied in 12 adults.

2. The results show an average increase in the calcium concentration of 4.4 per cent. Citric acid rose 10.7 per cent over the fasting levels. Inorganic phosphorus levels rose an average of 15 per cent. The greatest change was noted in the case of non-protein organic phosphorus which was of the order of 173 per cent increase.

3. Six grams of hydrolyzed calcium fructose diphosphate given orally did not cause a similar rise in non-protein organic phosphate in five cases.

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# THE CLINICAL DETERMINATION OF PROTEIN-BOUND IODINE[1]

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(Submitted for publication August 21, 1950; accepted, October 17, 1950)

The value of plasma protein-bound iodine (PBI) measurement in the evaluation of thyroid disease has been well established (1–7), but it has not yet assumed its deserved place in the clinical armamentarium because of the technical difficulties involved in distillation procedures. Modification of an alkaline-incineration technique (8) has resulted in the evolution of a method for PBI determination which is practicable for a clinical laboratory. It must be appreciated that extreme care will always be required in the quantitative measurement of 0.05 $\mu$gm. PBI/cc. plasma as well as in avoiding contamination.

## SOLUTIONS REQUIRED

All solutions should be made up in double-distilled water. If the amount of iodide in the water is still too high to give a satisfactory blank, it may be necessary to carry out these distillations with such reagents as alkali and permanganate.

All reagents used should be of highest purity obtainable. However, the iodine content in each instance must be judged from the blank values rather than from estimates shown on the labels. Often it is necessary to turn to a new source of supply or a different lot number in case of contamination.

*Zinc sulfate.* 100 grams of $ZnSO_4 \cdot 7\ H_2O$ per liter of solution.

*Sodium hydroxide.* 0.5 N is 20 grams of NaOH per liter of solution. The zinc sulfate and sodium hydroxide reagents should be so related that when 10.0 cc. of the former are diluted with 50–70 cc. of water and titrated with the latter, 10.8–11.2 cc. of the 0.5 N NaOH are required to produce a faint permanent pink color with phenolphthalein.

*Hydrochloric acid, 2 N.* 200 cc. C.P. concentrated HCl per liter of solution.

*Sulfuric acid, 7 N.* 196 cc. C.P. concentrated $H_2SO_4$ per liter of solution.

*Iodide standards.* Either NaI or KI of highest purity desiccator-dried may be used. If NaI is chosen, 118.1 mg. are dissolved in water and made to 1 l., if KI, 130.8 mg. These concentrated stock solutions contain 100.0 $\mu$gm. I/cc.

A more dilute stock is obtained by diluting 2 cc. of the above solution to 1 l. To prepare the standard of 0.04 $\mu$gm. I/cc. solution used in each determination, 10 cc. of this dilute stock are further diluted to 50 cc.

All of these solutions have been found to keep indefinitely at refrigerator temperatures, but it is preferable to make up the most dilute standard fresh every week or two.

*Ceric ammonium sulfate, 0.02 N.* 12.65 grams of the salt are dissolved in 500 cc. of water plus 230 cc. of 7 N $H_2SO_4$. When the solution is clear, it is made to 1 l. with water.

*Sodium arsenite, 0.1 N.* Dissolve 4.95 grams of $As_2O_3$ in 25 cc. of 4% (1N) NaOH, warming to hasten solution. This should be diluted with about 300 cc. of water and dilute sulfuric acid added until the solution is slightly acid to litmus paper (if the 7 N is used, about 4 cc. will be required). The solution is then made to 1 l. If sodium arsenite itself is used, 6.50 grams of $NaAsO_2$ should be dissolved in distilled water and diluted to 1.0 l. No acid or alkali is required with this salt.

## ANALYTICAL PROCEDURE

The steps involved can most conveniently be described under four headings: *1*) precipitation and washing of plasma proteins, *2*) drying and incineration of sample, *3*) dissolving iodide from the ash, *4*) colorimetric determination of iodide present.

*1) Precipitation (9) and washing of plasma proteins.* The blood sample, about 10 cc., is centrifuged and the plasma or serum drawn off. Duplicate aliquots of 1 cc. each are pipetted into 15 × 125 mm. Pyrex test tubes, diluted with 7 cc. of distilled water and 1.0 cc. of 10% zinc sulfate solution added. The contents of each tube are mixed with a glass stirring rod about 2 mm. in diameter, 1.0 cc. of 0.5 N sodium hydroxide is added and the solutions thoroughly mixed to insure even distribution of the alkali and complete precipitation of the proteins. Any material adhering to the rod can be removed by rubbing it with a rotary movement against the inside wall of the tube.

The tube and contents are centrifuged for 10 minutes to pack the precipitate and the supernatant fluid is poured off. Ten cc. of distilled water are added and the protein resuspended by means of the same stirring rod

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[1] This work was supported in part by a research grant from the Division of Research Grants and Fellowships, National Institutes of Health, U. S. Public Health Service and in part by a research grant from the Central Scientific Fund of the College of Medicine, State University of Iowa.

[2] Deceased June 21, 1949.

originally used. Stirring should be only sufficiently vigorous to distribute the zinc proteinate uniformly throughout the solution; if over-zealously carried out, the precipitate may be so finely divided that it cannot easily be centrifuged down. Centrifugation is again carried out and the supernatant discarded. This washing process is repeated two more times, making a total of three washings (the original precipitation is not included as a washing).

2) *Drying and incineration.* After the last supernatant solution has been poured off, 0.8 cc. of 4.0 N sodium carbonate is added and thoroughly stirred into the precipitate with the same stirring rod used in the washings. Removal of material clinging to the rod can be accomplished by rubbing as described previously, followed by 0.2 cc. of the 4.0 N $Na_2CO_3$ added dropwise down the rod. This last portion of alkali can easily be mixed with the sludge already present without use of the stirring rod. The tube is then placed in an oven set at 85–95° C. to drive off water. This usually requires 12–18 hours and is usually carried out overnight.

After thorough drying of the alkali and zinc proteinate, ashing is carried out by placing the tube in a muffle furnace for two and one half hours at $600 \pm 25°$ C. At the end of that time, the tube is removed and allowed to cool to room temperature.

3) *Dissolving iodide from the ash.* Two cc. of 2 N hydrochloric acid are added with due caution to avoid excessive effervescence. A glass rod is used to mix any unreacted ash with the acid, and 2.0 cc. of 7 N sulfuric acid and 3.0 cc. of water are added. The contents of the tube are stirred until the reaction appears to be finished, transferred to a clean tube and briefly centrifuged to pack the insoluble material.

4) *Determination of iodide (10).* For each tube a pair of colorimeter tubes is taken. To one of the pair is added 1.0 cc. of distilled water containing 0.04 μgm. of iodine as sodium or potassium iodide and to the other is added 1.0 cc. of water. A 3.0 cc. aliquot of the supernatant in the sample tube is pipetted into each colorimeter tube and 0.5 cc. of the sodium arsenite solution is added to each tube. Mixing should be carried out by some technique which avoids both loss of solution and contamination.

The colorimeter tubes are then placed in a well-stirred constant temperature water bath maintained at $39 \pm 0.1°$ C. for 10 minutes to come to temperature. The ceric ammonium sulfate solution should also be warmed in the bath. Since the actual determination of iodide is accomplished by measuring iodide catalysis of the rate of decolorization of yellow ceric ammonium sulfate by arsenious acid, the times at which colorimeter readings are made must be definitely set up. One cc. of the ceric solution must be added to each tube with adequate time allowance for reading the color change in the previous tube. This is accomplished in this laboratory by leaving 30-second interval between tubes and by setting up a [illegible] of only 12 tubes in a single series. Colorimeter gs are then made at six and 12 minutes after adding eric sulfate solution to each tube. If 30 seconds is adequate for making the reading, more time must be owed, and fewer determinations done in any one series. Since the measurement depends entirely upon the decrease in amount of color, the pipetting of the 1.0 cc. of ceric solution must be highly accurate, even though done rapidly. Mixing must again be rapid and thorough.

The Klett-Summerson readings (using blue filter No. 42) are plotted on semi-log paper against the time in minutes. Ordinary graph paper is suitable for instruments giving readings in terms of per cent light transmission. The colorimeter setting at zero time is obtained for any given batch of reagents by quickly reading a reagent blank immediately after addition of the ceric sulfate. The lines describing the two reaction rates are drawn using the points for 0, 6 and 12 minutes, as is shown in Figure 1 and then the horizontal line indicating times required to reach equal amounts of decolorization. The amount of iodide in the colorimeter tube without added iodide is calculated from the following equation, obtained from similar triangles (see Figure 1):

$$\mu\text{gm. I in colorimeter tube} = \frac{0.04 \times t_2}{t_1 - t_2}$$

$t_1$ = time in minutes at intercept of the rate of decolorization in tube containing no extra iodide with the designated horizontal.

$t_2$ = time in minutes at intercept of the rate of decolorization in tube containing added iodide with the designated horizontal.

The reagents used contain variable amounts of iodide and the total must be determined by blank analyses starting with the zinc sulfate and sodium hydroxide solutions and carried through the entire procedure, including the washings with 10 cc. of distilled water, addition of 1 cc. of sodium carbonate, drying, ashing, treatment with hydrochloric and sulfuric acids and the final colorimetric assay on 3 cc. aliquots just as with an unknown sample. The blank value calculated by the same formula shown previously has been 0.015 – 0.017 μgm. I/tube in this laboratory.

Since it is customary to express the values in terms of μgm./100 cc. plasma, the complete calculation would follow:

$$\text{Plasma PBI } \mu\text{gm. I/100 cc.} = 100 \times \frac{7}{3}\,(\text{I in final aliquot—blank I})$$

where the factor of 7/3 has been introduced to correct for the 3.0 cc. aliquot taken for colorimetric evaluation from the 7.0 cc. of acid supernatant from the ashed sample.

If so much iodide is present in the sample that decolorization is nearly complete in the second tube, with 0.04 μgm. of added I, the determination may be repeated using less than the 1.0 cc. of plasma usually called for. Computation of the results must be adjusted as necessary to cover this change.

## NOTES AND PRECAUTIONS

It must be emphasized that the various conditions explicitly detailed herein have been evolved after intensive study. Modifications to suit any other laboratory are quite in order, but they must be rigorously tested before being incorporated into the procedure.

*General.* Scrupulous care must be exercised to insure clean glassware. A detergent-soap mixture such as "Lakeseal" [3] is preferred for routine cleaning. This solution is rinsed off with tap water followed by double-distilled water. Contamination, especially from iodine and mercury, must be guarded against. Since elemental iodine is so volatile and in such prevalent use as an antiseptic, this comprises the greatest hazard. Tincture of iodine must be avoided as an antiseptic on the skin of the patient before the sample is drawn and of any persons carrying out the actual determination. If elemental iodine is being used in any chemical determination or histological procedure in the same laboratory, irregular and unpredictable contamination is almost inevitable. It is thus best to devote an entire small room to the iodine work if dependable results are to be obtained.

In contrast to this trouble which is usually avoidable, once the patient has been administered any form of organic iodide, such as Lipiodol, Priodax, Skiodan, Diodrast or tetraiodophenolphthalein, binding of the iodine compound onto plasma proteins (11–13) renders the determination without value. Within a few hours, the PBI rises, often reaching a peak greater than 350 μgm. %. Depending upon the nature and amount of the particular substance plus the method of its administration, elevated PBI values have been observed for six months to three years in the absence of any genuine metabolic disorder (2, 14). No satisfactory method exists for separating these iodine-containing compounds from the true PBI, even the extraction of "thyroxine iodine" with butyl alcohol (15). It is highly recommended that information as to this type of contamination be widely disseminated so that samples for PBI analysis will be drawn before rather than after a diagnostic procedure using any organic iodide.

*1) Precipitation and washing of plasma proteins.* The blood sample may be collected with an anticoagulant or may be allowed to clot. The advantages of the former are speed in obtaining the plasma and a larger volume of fluid resulting, and of the latter that no foreign material need be added to the blood. No difference has been found between plasma and serum PBI and the routine in this laboratory has been to use oxalated bottles prepared in advance by evaporating 0.1 cc. of 20% potassium oxalate solution in each. The labels should be distinctive to avoid exchange with bottles from other laboratories. If serum is to be used, a paraffined tube is advantageous to allow easy clot retraction.

The procedure of washing the zinc proteinate precipitate by resuspension in distilled water and centrifugation has been found by both chemical and radioiodine tracer studies [4] to remove between 99.0 and 99.5% of the inorganic iodine present. At plasma inorganic I levels of 2 μgm. % (for normal humans) to 100 μgm. % (on some Lugol's-treated patients), the residual amounts (about 0.01 to 1.00 μgm. %) are not large enough to interfere. However, thyrotoxic patients being given five drops t.i.d. often are up to 300 μgm. %, and one woman receiving 20 drops t.i.d. rose to 1120 μgm. %. Although it is of questionable therapeutic value to use such extremely high iodine doses, the situation occasionally comes up and the laboratory must constantly be on the lookout. If all iodine therapy is stopped at least four days prior to drawing a sample for PBI, the plasma inorganic iodide will return nearly to the normal level, so that three washings are adequate. If $I^{131}$ uptake by the thyroid gland is also to be measured, an even longer period of time will be required. It is felt that this type of precaution is better than increasing the number of washings of the protein precipitate.

*2) Ashing.*[5] It does not appear necessary to take any elaborate precautions to insure absolutely even distribution of the protein-sodium carbonate suspension on the wall of each tube. However, it is desirable to avoid leaving the entire contents at the bottom of the tube, since this may lead to large bubble formation during the drying. Often such a bubble will rise through the entire tube, carrying ahead of it considerable protein material and causing a loss of PBI.

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[3] Obtainable from the Finger Lakes Chemical Co., Etna, N. Y.

[4] The $I^{131}$ used in this investigation was supplied by the Oak Ridge National Laboratory on allocation from the Isotopes Division, U. S. Atomic Energy Commission.

[5] For the most consistent results possible, it has been found desirable to have a muffle furnace available for the exclusive use of the iodine laboratory. While awaiting replacement of a defective relay on our furnace, we attempted to share one in the institution's water laboratory. Random contaminations immediately became a serious problem until we could return to our own apparatus, although no source for the added iodide was ever found.

The ashing time and temperature have been found not critical, variations from two to three hours and from 575° to 625° C. causing insignificant differences in results. At temperatures above 650° C., softening of the Pyrex tubes occurs as well as loss of iodine. Although the ash in the recommended temperature range is gray rather than white, the incineration is adequate to put all of the organic iodine into extractable inorganic form. Below 500° C., there are so many products of incomplete ashing that the acid solution is yellow and substances are extracted which give a very high "iodine" value.

Each tube is used three or four times and then discarded in order to avoid excessive etching with possible loss of the sample.

3) *Dissolving iodide from ash.* The effervescence when the hydrochloric acid reacts with the ash is highly desirable in carrying off hydrogen sulfide formed from the sulfur-containing amino acids. If hydrogen sulfide remains when the ceric sulfate is added, insoluble ceric sulfide precipitates. Under the conditions described, this situation has never been observed.

4) *Colorimetric determination of iodide present.* All steps of this part of the procedure must be carried out with great care to reproduce accurately all conditions of volumes, time and temperature, since the measurement is made on a basis of iodide catalysis of a rate reaction. The actual conditions may be varied considerably, but those decided upon must be duplicated carefully from analysis to analysis if good agreement is to be had. This report has described the technique used at present in this laboratory on the experience of two years.

All mixing of solutions in this last phase must be accomplished by some technique which avoids both contamination and loss of solution, such as twirling each tube between the hands, flicking the bottom of each tube with several fingers in succession, or rapidly inverting each tube. The inversion or "flipping" procedure is preferred; this is carried out by grasping the colorimeter tube close to the open end between the thumb and first finger. The tube is inverted by raising it over one's head and then is rapidly returned. With a little practice one can achieve complete mixing of practically all miscible solutions in eight to 10 such inversions without losing a drop. This technique is especially valuable when the ceric sulfate solution must be mixed quickly and completely with the use of only one hand.

The method described earlier for carrying out the calculation was devised in order to rule out any possible effect of substances remaining in the final acid solution from the ash. Many nonvolatile compounds could conceivably be present in solution to alter the final colorimetric assay. Any such substance should affect the standard 0.04 µgm. of sodium iodide added to one aliquot as well as the unknown itself. Derivation of the computation

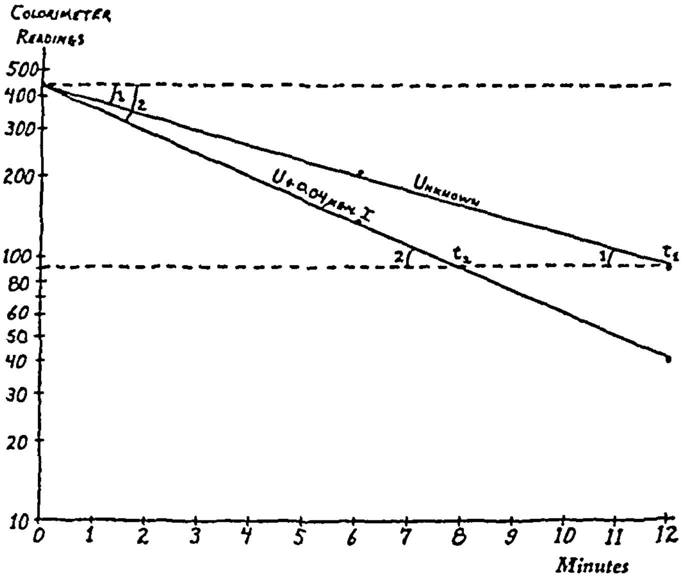

FIG. 1. METHOD OF PLOTTING RESULTS FOR CALCULATION OF PBI

$$\text{Tan} < 1 = \frac{\text{Initial colorimeter reading} - \text{final colorimeter reading}}{t_1}.$$

This is an expression of the decolorization of the U sample. Since the assumption is being made that the decolorization is due entirely to I,

$$\frac{\text{Difference in colorimeter readings}}{t_1} = \text{U } \mu\text{gm. I in sample}$$

$$\text{Difference in colorimeter readings} = \text{U} \times t_1$$

In similar fashion:

$$\text{Tan} < 2 = \frac{\text{Difference in colorimeter readings}}{t_2}$$

$$= \text{U} + 0.04\ \mu\text{gm. I}$$

$$\text{Difference in colorimeter readings} = (\text{U} + 0.04\ \mu\text{gm. I})t_2$$

Since the differences in colorimeter readings are identical,

$$\text{U} \times t_1 = (\text{U} + 0.04\ \mu\text{gm. I})t_2 = \text{U}t_2 + 0.04\ \mu\text{gm. I} \times t_2$$

$$\text{U}t_1 - \text{U}t_2 = 0.04\ \mu\text{gm. I} \times t_2$$

$$\text{U} = \frac{t_2 \times 0.04\ \mu\text{gm. I}}{t_1 - t_2}$$

The unknown also includes any iodide present in the reagents and a blank must be carried through as described in order to correct U for the blank.

TABLE I

*Comparison of PBI methods on 57 clinical plasmas*

| Patient | Plasma PBI | | | Patient | Plasma PBI | | |
|---|---|---|---|---|---|---|---|
| | Incin. | Distill. | Diff. | | Incin. | Distill. | Diff. |
| | (1) | (2) | (1-2) | | (1) | (2) | (1-2) |
| MR | 1.0 | 1.1 | −0.1 | RS | 6.4 | 6.8 | −0.4 |
| MN | 1.8 | 1.6 | +0.2 | GM | 6.8 | 7.4 | −0.6 |
| FMcE | 2.2 | 2.2 | 0.0 | OB | 6.9 | 7.0 | −0.1 |
| DD | 2.4 | 2.2 | +0.2 | EC | 7.6 | 8.4 | −0.8 |
| JS | 2.8 | 2.5 | +0.3 | WM | 7.8 | 7.4 | +0.4 |
| LP | 3.0 | 2.2 | +0.8 | SY | 9.1 | 9.5 | −0.4 |
| WM | 3.3 | 2.8 | +0.5 | EG | 9.5 | 8.8 | −0.7 |
| JP | 4.2 | 3.8 | +0.4 | JW | 9.8 | 9.3 | +0.5 |
| KF | 4.3 | 5.6 | −1.3 | VS | 9.8 | 9.9 | −0.1 |
| MB | 4.3 | 4.2 | +0.1 | AK | 9.9 | 8.1 | +1.8 |
| ED | 4.4 | 3.4 | +1.0 | EB | 10.0 | 13.2 | −3.2 |
| HC | 4.4 | 4.2 | +0.2 | SM | 10.5 | 10.0 | +0.5 |
| TH | 4.4 | 4.4 | 0.0 | HB | 11.1 | 12.3 | −1.2 |
| LB | 4.4 | 4.6 | −0.2 | JW | 11.4 | 10.1 | +1.3 |
| ID | 4.4 | 4.8 | −0.4 | AB | 12.0 | 9.0 | +3.0 |
| JM | 4.6 | 3.7 | +0.9 | JZ | 12.5 | 18.9 | −6.4 |
| OW | 4.6 | 4.9 | −0.3 | JT | 13.5 | 12.9 | +0.6 |
| CS | 4.8 | 4.0 | +0.8 | JH | 14.9 | 13.8 | +1.1 |
| NP | 4.8 | 4.1 | +0.7 | JR | 15.5 | 15.7 | −0.2 |
| GK | 4.9 | 3.6 | +1.3 | JR | 15.5 | 16.0 | −0.5 |
| EK | 5.0 | 4.8 | +0.2 | JZ | 16.0 | 17.8 | −1.8 |
| CM | 5.1 | 4.6 | +0.5 | SY | 16.2 | 16.7 | −0.5 |
| RM | 5.3 | 6.2 | −0.9 | DR | 19.5 | 16.4 | +3.1 |
| JS | 5.5 | 4.5 | +1.0 | IB | 19.9 | 17.9 | +2.0 |
| LP | 5.5 | 6.0 | −0.5 | ST | 21.3 | 20.4 | +0.9 |
| BT | 5.6 | 4.4 | −1.2 | FF | 21.5 | 28.0 | −6.5 |
| LN | 5.7 | 4.9 | −0.8 | JE | 27.8 | 26.3 | +1.5 |
| ES | 5.9 | 4.7 | +1.2 | RQ | 34.9 | 36.4 | −1.5 |
| MH | 5.9 | 5.6 | +0.3 | | | | |

$$\chi^2 = \Sigma\left(\frac{(1-2)^2}{2}\right) = 11.096 \text{ for 56 degrees of freedom}$$

Therefore, there is much greater than 99% probability that the results are the same.

tion formula using the 0, 6 and 12 minute readings is contained in the detailed heading accompanying Figure 1. Table I summarizes a series of 57 comparisons which we have carried out on duplicate clinical plasma samples between this PBI procedure and the distillation technique described some time ago (16). A comparison of means would have little significance because of the wide range of PBI values (1 to 35 µgm. %). Chi square analysis of the results was therefore carried out and shows clearly that there is no significant difference between the two series.

That the unorthodox procedure of manipulation of the decolorization readings does not itself introduce a significant error when carefully done is demonstrated by 43 plottings of the identical colorimeter data carried out in the course of communicating the details of this procedure to interested laboratories. The mean of the calculations of the amount of iodine per colorimeter tube was 0.0386 µgm., with a standard deviation of 0.0004. Similarly good agreement has been obtained when different persons have plotted out the same colorimeter readings.

The customary method of calculating by setting up a curve of standards and interpreting colorimeter readings of unknown solutions from this curve is much simpler, but suffers from the possibility of the presence of some substance which would enhance the decolorization or interfere with it. The two methods of computation have been compared on several series of clinical samples; the standard curve technique was consistently higher, by 0.8 µgm. % using the 6-minute readings and 1.3 µgm. % using 12-minute readings. Recovery of the added 0.04 µgm. of standard iodide was 99.3% and 100.0%, respectively. No basis for this difference can be offered at present. It is obvious that use of the simpler form of calculation will necessitate upward revision of the values to be considered normal by about 1 µgm. %.

### RECOVERY OF ADDED IODINE

As a test of the ability of the procedure to determine known amounts of iodine, standardized amounts of sodium iodide, thyroxine and thyroglobulin were added in triplicate to tubes containing precipitated and washed plasma proteins. Drying, ashing, HCl and $H_2SO_4$ elution and colorimetric determination of iodide present then followed. The results, shown in Table II, demonstrate adequate recovery of these substances in physiologically important amounts.

TABLE II

*Recovery of iodine-containing substances added to precipitated and washed proteins*

| Substance | Amount I added | Amount I recovered |
|---|---|---|
| | µgm. % | % |
| NaI | 2.0 | 97.5 |
| | 4.5 | 99.0 |
| | 5.0 | 108.9 |
| | 10.0 | 101.6 |
| | 20.0 | 102.3 |
| Thyroxine | 3.6 | 90.6 |
| | 7.7 | 98.0 |
| | 10.0 | 96.8 |
| Thyroglobulin | 2.5 | 99.9 |
| | 5.0 | 107.3 |
| | 15.0 | 94.2 |

## RESULTS WITH THE DETERMINATION

This procedure has been available to the staff of the University Hospitals for nearly two years. On the basis of the first year's experience, the technique was extensively revised during the summer of 1949. In the year since that time, 942 determinations have been carried out on 694 patients; the distribution of these is shown in Figure 2. A clustering of values between 3 and 7 μgm. % is apparent, but no sharp separations are present between "normal" and either hypo- or hyperthyroid individuals. From a physiological standpoint, such overlapping would be expected on the basis of all studies of normalcy. From the clinical point of view, it is important to establish some limits beyond which thyroid dysfunction can reasonably by expected.

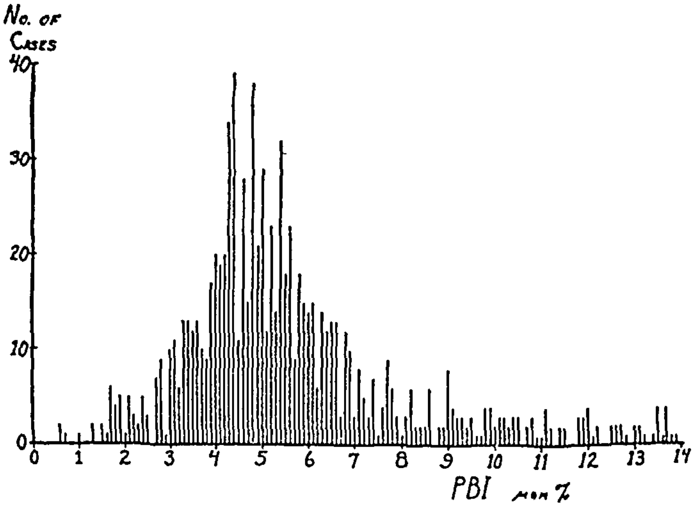

FIG. 2. DISTRIBUTION OF ONE YEAR'S DETERMINATIONS OF PBI ON CLINICAL PLASMA SAMPLES

In all, 942 results were obtained, but 50 values above 14 μgm. % have been omitted from the chart, since these were scattered up to 45 μgm. %.

In order to accomplish this, a group of samples has been assembled in Table III from medical students, resident staff, technicians and faculty, each person overtly appearing to be euthyroid. The table shows that a range of PBI values from 3.4 to 8.0 μgm. %, with a mean of 5.1, was obtained on 40 medical students who had BMR's between − 15 and + 15. A smaller group of 27 apparently normal individuals (staff, visitors, etc.) gave an identical mean, although no basal metabolism values were available for standardization. In view of these findings, it is probable that the range of normal, usually considered to be 4.0 to 8.0 μgm. %, must be extended downwards to 3.5 μgm. %. This conclusion is, of course, based partially on acceptance of the validity of the BMR. Since the usual error in this determination is on the plus side, one might argue that this error had operated in the three subjects whose PBI values were below 3.9 μgm. % and that they actually should be considered myxedematous. No answer to this is possible, except that no genuine symptoms of hypothyroidism were apparent in any of these individuals. It will require a great many carefully controlled determinations in the borderline PBI zone of 3.5–4.0 to settle this physiological as well as chemical problem.

TABLE III

*PBI values on 68 euthyroid subjects*

| Subjects | BMR | PBI | | |
|---|---|---|---|---|
| | Range | Mean | Range | σ |
| | | μgm. % | μgm. % | |
| 40 Medical students | +15 to −15 | 5.1 | 3.4–8.0 | 1.12 |
| 28 Miscellaneous | unknown | 5.1 | 3.6–6.6 | 0.79 |
| Entire series of 68 | | 5.1 | 3.4–8.0 | 1.00 |

It is highly possible that the upper limit should be 7.5, bringing the entire euthyroid group close to the range of 3.5–7.0 μgm. % as suggested by Riggs (2). The problems of overlap, as far as the PBI is involved, seem to be intensified in the lower, myxedema, portion, with very few euthyroid individuals above 7.5 or thyrotoxic below 8.5 μgm. %.

The histories on 86 of the patients on whom plasma PBI's had been run also contained results of BMR and $I^{131}$ uptake determinations. The data for PBI and BMR have been plotted in Figure 3, together with the overall clinical evaluation of each patient, proven by surgery, pathology or therapeutic response. The area between a BMR of + 15 and − 15 and between 3.5 and 8.0 for PBI has been marked off as normal. It can be seen that one value in the myxedema group would be considered normal (at 3.5 μgm. %), one thyrotoxic was well in the normal range, and one very high value (14.3 μgm. %) was obtained on a subject with a non-toxic, diffusely enlarged goiter. In contrast, several BMR determinations in each category were at considerable variance with the general clinical opinion. The greater reliability

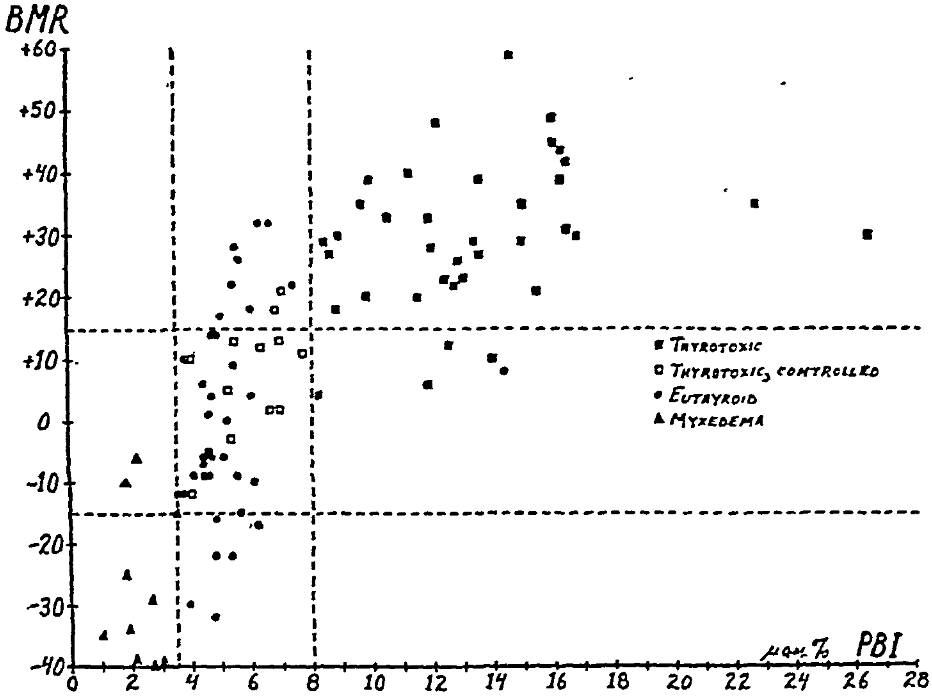

FIG. 3. COMPARISON OF PBI, BMR AND ENTIRE CLINICAL EVALUATION OF 86 PATIENTS

The 12 values represented by hollow squares were performed on previously thyrotoxic subjects, following successful management with surgery, $I^{131}$, or propylthiouracil. All patients had been on therapy at least one month, which should be an adequate length of time for the metabolic rate to return to normal (17).

of the PBI over the BMR has previously been noted by several investigators (2, 3, 18) and may be attributed, at least in part, to the greater objectivity of the chemical determination.

In discussing the advantages of this procedure, it may simply be stated that, in general, distillation techniques, although having a wider application because of greater flexibility, require such close attention during the digestion and distillation steps as to be poorly suited for routine clinical evaluation. On the other hand, incineration can be automatically regulated, making the final colorimetry the only stage requiring personal concentration. No attempt has been made in this laboratory to determine the maximum number of determinations possible per day, since successful routine analysis depends much more upon consistent performance week after week. It may conservatively be estimated that 15–20 samples can be analyzed in duplicate every two days by a single individual.

## SUMMARY

1. A method has been presented for determination of plasma or serum protein-bound iodine (PBI) using high-temperature incineration in the presence of alkali. The distillation step, requiring close individual attention, has been eliminated. The final determination of inorganic iodide is performed colorimetrically by measuring the catalysis of arsenious acid decolorization of a ceric sulfate solution.

2. Data are given for considering the range of normal to be 3.5 to 8 μgm. %.

3. Examination of two years' experience with this PBI analysis shows it to be an objective test of greater dependability than the BMR.

## ACKNOWLEDGMENT

The help of Dr. C. D. Janney of the Radiation Research Laboratory and of Drs. W. H. Ames and J. A. Clifton of the Department of Internal Medicine in carrying out various portions of this work is gratefully acknowledged. Dr. F. W. Schueler aided in the statistical analysis.

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# STUDIES OF THE ANTIDIURESIS OF QUIET STANDING: THE IMPORTANCE OF CHANGES IN PLASMA VOLUME AND GLOMERULAR FILTRATION RATE[1]

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(Submitted for publication August 21, 1950; accepted, October 17, 1950)

## INTRODUCTION

It has long been known that when a man stands motionless, urine flow (1–13) and the excretion of chloride (2, 3, 5, 7, 11) and of sodium (5, 13) decrease markedly. Simultaneously, the plasma contracts in volume and increases in concentration (8, 10, 14–17), while the blood flow through the kidneys (9, 10, 18, 19) and other abdominal viscera (20) diminishes, and the rate of glomerular filtration of plasma and its contained sodium is reduced (6, 9, 10, 13, 18, 21).

There is some evidence that release of the posterior pituitary antidiuretic hormone may be responsible for the retention of water (11) but the mechanism of the retention of sodium and the stimulus or stimuli initiating it remain obscure. Some renal physiologists would place greatest emphasis on the reduction in glomerular filtration rate which occurs during standing, and the resulting decreased load of sodium presented to the tubules (5, 22, 23). It is not likely that an increase in the renal venous pressure (24, 25) plays an important role in the antidiuresis of quiet standing, because when a subject is tilted from the supine to the erect position, pressure in the inferior vena cava at the level of the kidneys does not rise to the level reported by Blake and associates (25) to cause a reduction in the urine flow, and the renal arterio-venous pressure gradient does not change materially (26).

It has also been suggested that the reduction of total blood volume initiates a chain of events which culminates in decreased excretion of sodium (27–29). This might be accomplished through changes in glomerular filtration and/or tubular reabsorption of sodium. Some workers believe that this mechanism may operate in such diverse situations as hemorrhage, hypoproteinemic edema and congestive heart failure, as well as in quiet standing (28, 30).

The present studies were undertaken to investigate the relationship of changes in total plasma volume and glomerular filtration rate to the renal excretion of sodium during quiet standing.

## METHODS

All subjects were healthy young men, presumably able to empty their bladders without catheterization. Relatively high urine flows and long periods minimized any errors in collection. A fairly constant rate of excretion of sodium was achieved by the ingestion of 200 ml. of hypotonic (0.2%) saline solution per hour for several hours (31), and by preliminary rest supine in bed for at least one hour, before observations were begun. Subjects continued to drink saline at the same rate during the experimental period. In five experiments (Nos. 1, 10, 11, 13, 14) the glomerular filtration was estimated from the clearance of mannitol, using the single injection method with a falling plasma concentration (32). Previous observations had demonstrated that one could maintain a constant diuresis of salt and water for several hours under these conditions (33). In six cases (Nos. 4–6, 8, 9, 15) a constant infusion of mannitol was used, and in the remaining experiments a constant infusion of inulin. Renal plasma flow was calculated from the clearance of sodium para-aminohippurate administered as a constant infusion in normal saline. Mannitol in serum and urine was analyzed by the method of Smith (34), modified according to Elkinton (35). Inulin and para-aminohippurate were determined using methods described by Goldring and Chasis (36). Concentrations of sodium in serum and urine were measured with a flame photometer. Acute changes in plasma volume (PV) were calculated

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[1] Presented in part before the National Meeting of the American Federation for Clinical Research, May, 1950.

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TABLE I

*Changes in sodium excretion, plasma volume and renal function with quiet standing*

| Experiment no. and subject | Period | Duration | Urine flow | Sodium excretion | Mannitol clearance | Inulin clearance | Average serum Na | Sodium filtered | $\frac{E^*}{F}$ Na | % Change in plasma volume from control | PAH clearance | Mannitol excreted |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *min.* | *ml./min.* | *mEq./min. per 1.73 m²* | *ml./min. per 1.73 m²* | *ml./min. per 1.73 m²* | *mEq./L* | *mEq./min. per 1.73 m²* | | | *ml./min. per 1.73 m²* | *mM/min.* |
| 1 ERP | 1-supine | 67 | 4.18 | 0.159 | 85.6 | — | 136.5 | 11.5 | 0.014 | 0 | — | 0.43 |
| | 2-standing | 60 | 1.97 | 0.0714 | 76.0 | — | 136.5 | 10.21 | 0.007 | −13 | — | 0.25 |
| | 3-supine | 61 | 0.74 | 0.0197 | 61.3 | — | 136.5 | 8.27 | 0.002 | − 4 | — | 0.14 |
| 2 FHE | 1-supine | 30 | 8.1 | 0.266 | — | 108.3 | 144.0 | 15.5 | 0.017 | 0 | 599 | — |
| | 2-supine | 32 | 5.0 | 0.305 | — | 99.4 | 144.5 | 14.3 | 0.021 | − 6 | 556 | — |
| | 3-standing | 25 | 1.6 | 0.218 | — | 85.0 | 145.0 | 12.2 | 0.018 | −10 | 526 | — |
| | 4-supine | 38 | 2.4 | 0.252 | — | 86.0 | 144.5 | 12.3 | 0.020 | − 8 | 589 | — |
| 3 ASR | 1-supine | 30 | 3.90 | 0.181 | — | — | — | — | — | — | 722 | — |
| | 2-supine | 30 | 3.93 | 0.155 | — | — | — | — | — | 0 | 585 | — |
| | 3-standing | 30 | 1.55 | 0.096 | — | — | — | — | — | — | 526 | — |
| | 4-standing | 30 | 0.57 | 0.073 | — | — | — | — | — | − 7 | 548 | — |
| | 5-supine | 30 | 0.77 | 0.081 | — | — | — | — | — | — | 743 | — |
| | 6-supine | 31 | 1.16 | 0.090 | — | — | — | — | — | 0 | 787 | — |

* $\frac{\text{Sodium excreted}}{\text{Sodium filtered}}$.

from changes in the hemoglobin (Hgb) and hematocrit (Hct) of venous blood drawn without stasis, using formula:

$$\frac{PV_2}{PV_1} = \frac{Hgb_1}{Hgb_2} \times \frac{1 - Hct_2}{1 - Hct_1},$$

where the numbers in subscript indicate the values at the beginning and the end of a given period, respectively. Samples of blood for analysis were drawn at the beginning and at the end of each clearance period. The rate of filtration of sodium was calculated as the product of the average concentration of serum sodium and glomerular filtration rate, corrected for an assumed serum water concentration of .93 and a Donnan factor for sodium of 0.95. The fraction of filtered sodium rejected by the tubules and appearing in the urine was calculated as the fraction:

$$\frac{\text{Na excreted}}{\text{Na filtered}}, \text{ (E/F Na)}.$$

## RESULTS

### *Quiet Standing Alone (Table I)*:

In three subjects the renal effects of standing quietly for 25 to 60 minutes were quite typical of

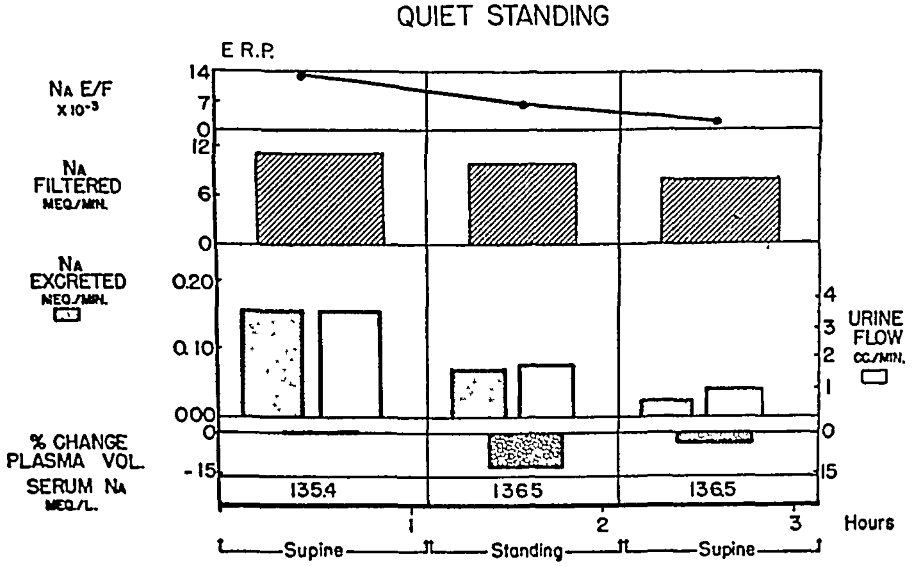

FIG. 1. EFFECT OF ONE HOUR OF QUIET STANDING

Note prolonged antidiuresis and the reduction in sodium excretion, associated with a significant contraction of plasma volume.

TABLE II

*Effect of infusions of serum albumih during quiet standing*

| Experiment no. and subject | Period | Duration | Urine flow | Sodium excretion | Mannitol clearance | Inulin clearance | Average serum Na | Sodium filtered | $\frac{E^*}{F}$ Na | % Change in plasma volume from control | PAH clearance | Mannitol excreted |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *min.* | *ml./min.* | *mEq./min. per 1.73 m²* | *ml./min. per 1.73 m²* | *ml./min. per 1.73 m²* | *mEq./L* | *mEq./min. per 1.73 m²* | | | *ml./min. per 1.73 m²* | *mM/min.* |
| 4<br>GG | 1-supine | 57 | 9.39 | 0.150 | 86.5 | — | 132.5 | 11.4 | 0.013 | 0 | 524 | 0.52 |
| | 2-standing<br>300 ml. 25% albumin in 34 min. | 49 | 4.59 | 0.103 | 79.6 | — | 134.7 | 10.6 | 0.010 | + 6 | 474 | 0.43 |
| | 3-supine | 41 | 4.15 | 0.043 | 73.4 | — | 135.9 | 9.9 | 0.004 | +26 | 528 | 0.37 |
| 5<br>IT | 1-supine | 33 | 5.15 | 0.223 | 79.0 | — | 141.2 | 11.0 | 0.020 | 0 | 498 | 0.53 |
| | 2-standing<br>750 ml. 4% albumin in 34 min. | 44 | 4.54 | 0.194 | 72.0 | — | 139.6 | 10.0 | 0.019 | + 4 | 479 | 0.52 |
| | 3-standing | 38 | 2.71 | 0.012 | 68.3 | — | 138.9 | 9.4 | 0.012 | + 3 | 462 | 0.54 |
| 6<br>ASR | 1-supine | 35 | 3.51 | 0.180 | 92.0 | — | — | 12.9† | 0.014 | 0 | — | 0.52 |
| | 2-supine | 33 | 5.24 | 0.283 | 97.0 | — | — | 13.6 | 0.021 | 0 | — | 0.56 |
| | 3-standing<br>750 ml. 4% albumin in 30 min. | 37 | 3.51 | 0.208 | 82.3 | — | — | 11.5 | 0.018 | + 3 | — | 0.47 |
| | 4-standing | 32 | 2.75 | 0.182 | 84.5 | — | — | 11.8 | 0.015 | + 7 | — | 0.48 |
| | 5-supine | 37 | 1.76 | 0.099 | 74.5 | — | — | 10.4 | 0.010 | +10 | — | 0.42 |
| 7<br>FHE | 1-supine | 25 | 7.40 | 0.451 | — | 96 | 145.8 | 13.9 | 0.032 | 0 | 625 | — |
| | 2-supine | 32 | 4.37 | 0.533 | — | 92 | 145.0 | 13.2 | 0.040 | + 4 | 642 | — |
| | 3-supine | 30 | 5.33 | 0.500 | — | 90 | 144.5 | 12.9 | 0.039 | + 6 | 675 | — |
| | 4-standing<br>750 ml. 4% albumin in 35 min. | 42 | 2.02 | 0.240 | — | 83 | 145.0 | 11.9 | 0.020 | + 7 | 578 | — |
| | 5-standing | 32 | 1.41 | 0.109 | — | 99.5 | 144.8 | 14.3 | 0.008 | +15 | 730 | — |

* $\frac{\text{Sodium excreted}}{\text{Sodium filtered}}$.

† Serum concentration of Na assumed to be 140 mEq./L.

those described by others (Figure 1). Renal plasma flow, the filtration and excretion of water and sodium, and the tubular rejection of sodium (E/F Na) all decreased. These changes lasted for some time after resumption of the supine position. Plasma volume decreased by 4 to 13%, but [as previously reported for serum chloride and bicarbonate (14)] the concentration of serum sodium did not change significantly.

*Quiet Standing with Albumin Infusion (Table II):*

To determine whether this sequence of events would be altered if the contraction of plasma volume were prevented, four subjects were given infusions of salt-poor human serum albumin[6] when they stood up. In three experiments, 750 ml. of isosmotic albumin (4% solution in normal saline) were infused, while in one case, 300 ml. of a 25% solution were injected. The infusions were given over a period of 30 to 35 minutes. A typical result is shown in Figure 2. Although in all cases total plasma volume increased slightly, in no instance was the antidiuresis of salt and water, which ordinarily accompanies quiet standing, prevented; and in every case a fall in the glomerular filtration of sodium and in E/F Na occurred. In experiment 7 (Figure 2), the excretion of sodium continued to decline during the last period despite an increase above control levels in both plasma volume and filtered sodium. Changes in renal plasma flow tended to parallel changes in the rate of glomerular filtration.

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[6] Serum albumin used in this study was prepared by the American Red Cross from blood of volunteer donors. The conclusions are those of the authors and do not necessarily reflect the policy of the National Blood Program of the American Red Cross.

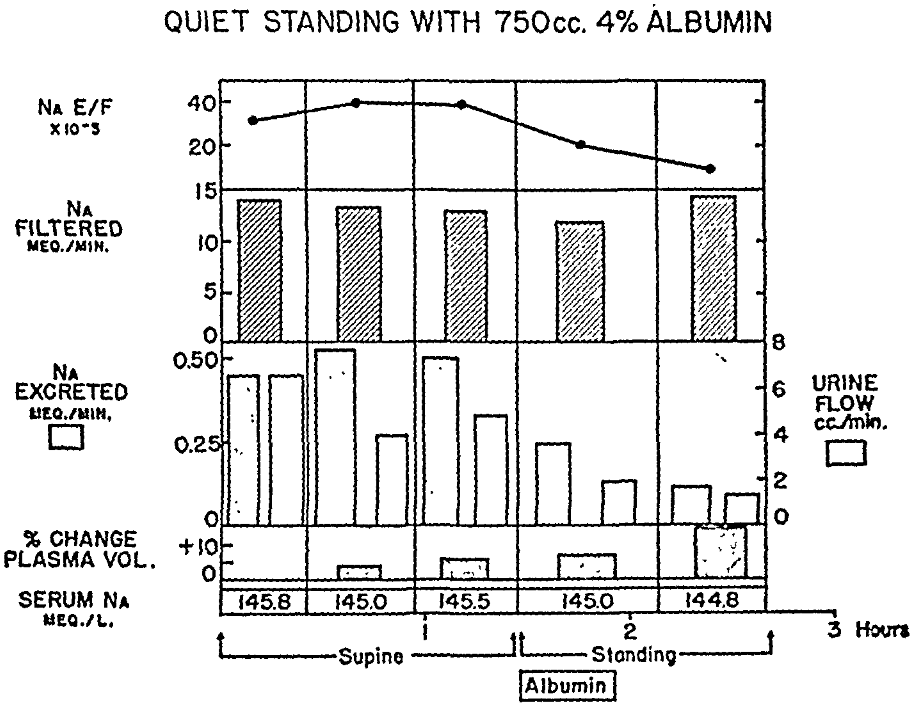

**Fig. 2**

Albumin infusion prevented the expected contraction of plasma volume during the period of quiet standing, but did not prevent the usual antidiuresis. Note that excretion of sodium and water continued to fall in the last period, although the rate of sodium filtration increased.

*Effects of Infusions of Hypertonic Saline (Tables III and IV):*

It is well known that the sodium diuresis following infusions of hypertonic saline is accompanied by an increase in the concentration of sodium in the serum and expansion of the volume of the plasma and extracellular fluid. In two experiments (Table III), infusions of hypertonic saline (6%) given to recumbent subjects sharply augmented the excretion of sodium, with (No. 9) or

TABLE III

*Effect of rapid infusions of hypertonic sodium chloride in supine subjects*

| Experiment no. and subject | Period | Duration | Urine flow | Sodium excretion | Mannitol clearance | Inulin clearance | Average serum Na | Sodium filtered | $\frac{E^*}{F}$ Na | % Change in plasma volume from control | PAH clearance | Mannitol excreted |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *min.* | *ml./min.* | *mEq./min. per 1.73 m²* | *ml./min. per 1.73 m²* | *ml./min. per 1.73 m²* | *mEq./L* | *mEq./min. per 1.73 m²* | | | *ml./min. per 1.73 m²* | *mM/min.* |
| 8<br>McG | 1-supine | 39 | 7.3 | 0.224 | 119 | — | 132.0 | 15.55 | 0.014 | — | 485 | 0.62 |
| | 2-supine | 62 | 9.2 | 0.282 | 129 | — | 135.9 | 17.35 | 0.016 | — | 536 | 0.68 |
| | 3-supine<br>400 ml. 6% saline in 23 min. | 47 | 6.6 | 0.870 | 120 | — | 141.6 | 16.94 | 0.051 | — | 444 | 0.62 |
| | 4-supine | 48 | 7.4 | 1.11 | 118 | — | 143.8 | 16.80 | 0.066 | — | — | 0.62 |
| 9<br>BR | 1-supine | 40 | 7.13 | 0.135 | 120 | — | 131.7 | 15.65 | 0.009 | — | 636 | 0.48 |
| | 2-supine | 39 | 10.40 | 0.125 | 107 | — | 133.5 | 14.14 | 0.009 | — | 651 | 0.43 |
| | 3-supine<br>400 ml. 6% saline in 39 min. | 52 | 3.75 | 0.287 | 116 | — | 139.0 | 15.95 | 0.018 | — | 725 | 0.47 |
| | 4-supine | 34 | 3.92 | 0.520 | 128 | — | 141.7 | 17.95 | 0.029 | — | 902 | 0.52 |

$* \frac{\text{Sodium excreted}}{\text{Sodium filtered}}$.

TABLE IV

*Effect of infusions of hypertonic sodium chloride during quiet standing*

| Experiment no. and subject | Period | Duration | Urine flow | Sodium excretion | Mannitol clearance | Inulin clearance | Average serum Na | Sodium filtered | $\frac{E^*}{F}$ Na | % Change in plasma volume from control | PAH clearance | Mannitol excreted |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *min.* | *ml./min.* | *mEq./min. per 1.73 m²* | *ml./min. per 1.73 m²* | *ml./min. per 1.73 m²* | *mEq./L* | *mEq./min. per 1.73 m²* | | | *ml./min. per 1.73 m²* | *mM/min.* |
| 10 ASR | 1-supine | 49 | 3.27 | 0.296 | 108.0 | — | 140.5 | 14.95 | 0.020 | 0 | — | 0.31 |
| | 2-standing | 55 | 1.36 | 0.053 | 70.6 | — | 136.5 | 9.50 | 0.006 | −21 | — | 0.18 |
| | 3-standing<br>400 ml. 3% saline in 30 min. | 63 | 0.75 | 0.023 | 64.9 | — | 142.0 | 9.10 | 0.003 | +1 | — | 0.14 |
| 11 ERP | 1-supine | 50 | 4.00 | 0.438 | 88.3 | — | 137.2 | 12.1 | 0.036 | — | — | 0.50 |
| | 2-standing<br>400 ml. 4% saline in 25 min. | 75 | 2.37 | 0.266 | 67.0 | — | 142.0 | 9.5 | 0.028 | — | — | 0.23 |
| | 3-supine | 51 | 2.12 | 0.315 | 77.0 | — | 141.9 | 10.9 | 0.029 | — | — | 0.13 |
| 12 FHE | 1-supine | 31 | 4.68 | 0.499 | — | 97.0 | 146.0 | 14.05 | 0.035 | 0 | 646 | — |
| | 2-supine | 39 | 5.57 | 0.554 | — | 94.0 | 145.6 | 13.58 | 0.040 | − 1 | 638 | — |
| | 3-standing<br>400 ml. 6% saline in 35 min. | 45 | 2.04 | 0.443 | — | 88.0 | 149.7 | 13.05 | 0.034 | 0 | 652 | — |
| | 4-standing | 30.5 | 1.72 | 0.439 | — | 83.0 | 152.1 | 12.53 | 0.035 | 0 | 584 | — |
| 13 AVG | 1-supine | 48 | 5.10 | 0.666 | 98.0 | — | 136.5 | 13.3 | 0.050 | — | — | 0.28 |
| | 2-standing<br>400 ml. 6% saline in 25 min. | 71 | 3.66 | 0.622 | 74.5 | — | 143.0 | 10.6 | 0.059 | — | — | 0.21 |
| | 3-standing | 55 | 2.18 | 0.412 | 49.0 | — | 143.5 | 7.0 | 0.059 | — | — | 0.20 |
| 14 FHE | 1-supine | 54 | 5.65 | 0.627 | 94.5 | — | 139.1 | 13.1 | 0.046 | — | — | 0.54 |
| | 2-standing<br>400 ml. 6% saline in 37 min. | 63 | 4.92 | 0.743 | 86.3 | — | 146.5 | 12.6 | 0.059 | — | — | 0.32 |
| | 3-supine | 53 | 4.15 | 1.00 | 97.8 | — | 152.5 | 14.9 | 0.067 | — | — | 0.24 |
| 15 DM | 1-supine | 40 | 2.50 | 0.210 | 100.0 | — | 138.9 | 13.80 | 0.015 | 0 | | 0.62 |
| | 2-supine | 44 | 3.07 | 0.238 | 99.0 | — | 137.6 | 13.52 | 0.017 | 0 | 432 | 0.55 |
| | 3-standing | 41 | 4.83 | 0.468 | 92.0 | — | 140.0 | 12.80 | 0.036 | +16 | 522 | 0.49 |
| | 400 ml. 6% saline in 26 min. | | | | | | | | | | 613 | |
| | 4-standing | 39 | 2.82 | 0.356 | 100.0 | — | 145.1 | 14.40 | 0.025 | +11 | 437 | 0.50 |
| 16 ASR | 1-supine | 30 | 3.80 | 0.200 | — | 100. | 146.0 | 16.60 | 0.014 | 0 | 774 | — |
| | 2-supine | 31 | 3.64 | 0.259 | — | 89. | 145.5 | 14.70 | 0.020 | − 2.5 | 697 | — |
| | 3-standing<br>400 ml. 7½% saline in 40 min. | 45 | 3.04 | 0.532 | — | 105. | 153.5 | 18.30 | 0.033 | +18 | 865 | — |
| | 4-standing | 29 | 3.80 | 0.852 | — | 108. | 157.0 | 19.1 | 0.051 | +17 | 940 | — |

* $\frac{\text{Sodium excreted}}{\text{Sodium filtered}}$.

without (No. 8) increasing appreciably the glomerular filtration of sodium or the renal plasma flow. In seven other experiments (Table IV), therefore, the hypertonic saline was infused during a period of standing, in an attempt to observe how the plasma volume, the concentration of sodium in the serum, and the rate of glomerular filtration of sodium were related to the excretion of sodium when large loads of salt were administered to subjects assuming the erect posture.

bumin. However, it must be emphasized that such infusions control only the *total volume* of the circulating blood and do not necessarily alter its *distribution* during quiet standing. Blood pooled in certain peripheral areas may not contribute to the effectiveness of the circulation, and in this sense the "effective blood volume" (42) may be reduced even when the total blood volume remains unchanged. Standing erect decreases both the total and effective volumes of the circulation by increasing the capillary filtration and the pooling of blood in the limbs. When these disturbances are minimized by immersing the body in water up to the level of the heart or by applying pneumatic occluding cuffs to the legs, the antidiuresis of the erect posture is modified (8, 12). It would seem, therefore, that if the kidneys react to changes in some function of the volume of the circulating blood when a man stands erect, they must react more to changes in its effective distribution than in its total volume.

The view that the antidiuresis of quiet standing is mediated primarily through a change in renal hemodynamics was first advanced by Hooker, who invoked the narrowed pulse pressure in the renal artery as a cause for diminished urine flow (4, 43). White, Rosen, Fischer, and Wood (5) ascribed the increase in the excretion of water and electrolytes during recumbency mainly to an increase in glomerular filtration rate. On the other hand, Ni and Rehberg (6), using the clearance of exogenous creatinine as a measure of filtration rate, came to the conclusion that postural changes in urine flow resulted from changes in both tubular reabsorption and glomerular filtration. In the present experiments, reductions in the urinary excretion of sodium during quiet standing were usually accompanied by significant alterations in both the filtration rate and the tubular rejection fraction of sodium (E/F Na). However, when infusions of albumin and hypertonic saline were given to subjects standing erect, a fall in sodium excretion was not always accompanied by a fall in the glomerular filtration of sodium (No. 7), nor did a small decrease in the filtration of sodium always result in diminished excretion of sodium (Nos. 14, 15).

These experiments appear to shed light on the nature of the renal response to loads of hypertonic saline, as well as to the upright posture. It has been suggested that the urinary excretion of sodium is normally regulated through fluctuations in its glomerular filtration (22, 23, 44). Selkurt and Post (45) reported that when dogs are infused with hypertonic saline, the excretion of sodium varies as the filtered load, and Dean and McCance (46) concluded that during hypertonic saline infusions in human subjects, "changes in glomerular filtration must underlie, and in part determine, the observed changes in urine flow." It is therefore noteworthy that in experiments 8, 14, and 15, when 6% saline was infused, the excretion of sodium increased without a concomitant increase in filtered load (but not without an increase in plasma volume).

The use of mannitol in these experiments may be questioned on the grounds that since excreted mannitol may sweep salt into the urine (22, 44, 47, 48) sodium excretion might necessarily appear to be related to the excretion, and hence the filtration of mannitol. This consideration enhances the significance of such discordant changes in the filtration and excretion of sodium as were observed in experiments 8, 14, and 15. Nevertheless, even under the strenuous conditions of these experiments it was difficult to change greatly the excretion of sodium in a direction opposite to simultaneous changes in sodium filtration. This does not necessarily prove that excretion is dependent upon filtration, since they may both be related through a third controlling factor.

Except in cases where the changes are very large, the errors inherent in present measurements of glomerular filtration prevent any accurate apportionment of responsibility to tubules or glomeruli for alterations in sodium excretion. Each milliliter of glomerular filtrate contains approximately 0.14 mEq. of sodium. An unavoidable 5% error in the estimation of glomerular filtration may therefore involve as much as 1 mEq./min. of sodium.

The renal response to salt loads is conditioned in part by the rise in concentration of sodium in the extracellular fluid. When infusions of hypertonic saline are given to thirsted subjects, the rate of sodium excretion rises, but drops sharply when the serum sodium is suddenly restored to normal by the ingestion of a large quantity of water (49). Some workers have concluded that when an infusion of hypertonic saline is given, "changes in sodium excretion are invoked by differences between extracellular and intracellular osmotic pressures" (50). The present studies show that even when the concentration of sodium in the serum rises significantly, it is sometimes possible to prevent or reverse the usual renal response by standing upright. A similar effect from abdominal compression has been reported recently (51).

The present data are consistent with the pluralistic view that alterations in both the composition and the effective distribution of the circulating plasma are important in initiating acute renal adjustments in sodium excretion. These adjustments may be accomplished by appropriate changes in the glomerular filtration or the tubular reabsorption of sodium, or both.

## CONCLUSIONS

1. The antidiuretic reaction to standing does not require a contraction of the total plasma volume.
2. It is possible to reverse this reaction by administration of hypertonic saline without increasing the rate at which sodium is filtered; but in so doing, the plasma volume is expanded.
3. The renal response to sodium loads under these conditions is not dependent solely upon alterations in the glomerular filtration of sodium or in the concentration of sodium in the serum.

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# STUDIES IN GLYCINE-2-$C^{14}$ METABOLISM IN MAN. I. THE PULMONARY EXCRETION OF $C^{14}O_2$[1]

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(Submitted for publication September 5, 1950; accepted, October 17, 1950)

## INTRODUCTION

There has been considerable discussion regarding the use of $C^{14}$ in clinical investigation, particularly from the standpoint of retention in the body of a long lived (5600 years) radioactive isotope. There is evidence from animal investigations that following the intravenous injection of simple $C^{14}$ labeled compounds occurring in normal biochemical processes most of the radioactivity will be excreted as $C^{14}O_2$ within a few days (1–5). The rate of excretion in mice via the lungs and the rate of loss of $C^{14}$ from the soft tissues and bone when this isotope is administered as glycine-2-$C^{14}$ has been determined; most of the radioactivity was eliminated in the breath in the first two days and the slowest tissue component was found to have a half time of approximately 10 days (6). There is no method available for extrapolation of these animal data to man. The present study was designed to measure the rate of pulmonary excretion of $C^{14}$ as $C^{14}O_2$ when administered as glycine-2-$C^{14}$.

## METHODS

Methyl labeled glycine was prepared by chlorination and amination of acetic acid-2-$C^{14}$ and had a specific activity of 12.2 microcuries/mg. of glycine (7). A series of four patients was given approximately 100 microcuries of glycine-2-$C^{14}$ (8 mg.) intravenously in 10 ml. distilled water for the purpose of the determination of the red blood cell life (8), since previous investigations with radio-iron had indicated that many patients with polycythemia and chronic leukemia might have red blood cells with a shorter than normal life span (9). Frequent breath samples were taken by a device (see Figure 1) designed to collect the expired air in a rubber balloon for a measured period of time. The balloon was transferred to a bubbler system (see Figure 2) and the expired air drawn through a 1N sodium hydroxide solution. The carbon dioxide thus absorbed was precipitated as $BaCO_3$ by $NH_4NO_3$ and $BaCl_2$. The barium carbonate was filtered, dried at 110° C and weighed to determine the amount of $CO_2$ excreted per minute. The samples were counted with a proportional counter[2] where the specific activity was greater than 10 dis./min./mg. $BaCO_3$; those with a lower activity were measured in an ionization chamber. These instruments were calibrated with the current Oak Ridge $C^{14}$ standard millicurie by specially prepared samples.

Inasmuch as the rate of pulmonary excretion of $C^{14}$ when administered in this form was not known at the start of these studies, the patients were placed in an oxygen tent although no oxygen was supplied, so that the expired air would pass through the soda-lime filter and thus absorb the carbon dioxide. In addition a vacuum pump continually exhausted the air from inside the tent to the outside of the building. These precautions were maintained for approximately 36 hours.

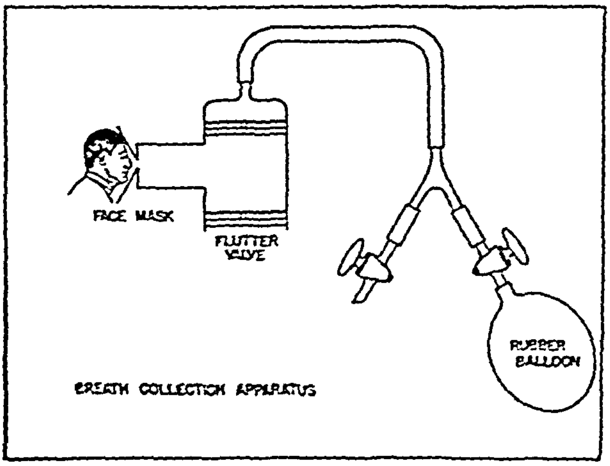

FIG. 1.
BREATH COLLECTION APPARATUS
(Note: not drawn to scale.)

## RESULTS

Figure 3 shows the specific activity as a function of time of the expired carbon dioxide as $BaCO_3$. The maximum specific activity of the expired breath occurred within the first hour following the injection. A measurable amount of radio-

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[1] This work was supported in part by the Atomic Energy Commission.

[2] Nucleometer—Manufactured by Radiation Counter Laboratory, Chicago, Illinois.

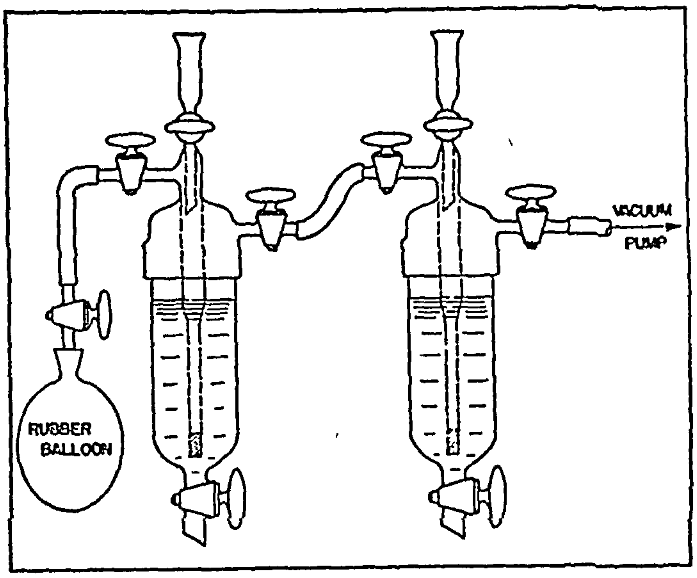

FIG. 2.
APPARATUS FOR CARBON DIOXIDE ABSORPTION

activity was present within the first 10 minutes following the injection. Figure 4 shows the calculated cumulative $C^{14}$ excretion as determined from the amount of $CO_2$ excreted per minute and the rate constants obtained from Figure 1.

The rate of excretion of $C^{14}$ when administered in this manner may be described in terms of three rate processes: the first having a half time of two to four hours; the second, 16 to 40 hours; and the third, seven to 14 days. Table I shows the half times and the percentages of $C^{14}$ excreted through each of the three rate processes for the four patients.

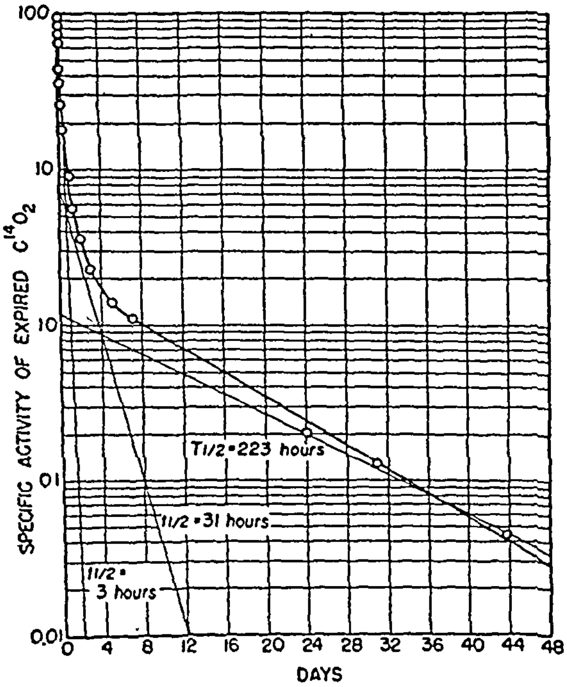

FIG. 3.
THE SPECIFIC ACTIVITY OF THE EXPIRED BREATH AS A FUNCTION OF TIME, IN PATIENT 1, IN DISINTEGRATIONS/MIN./MG. OF $BaCO_3$

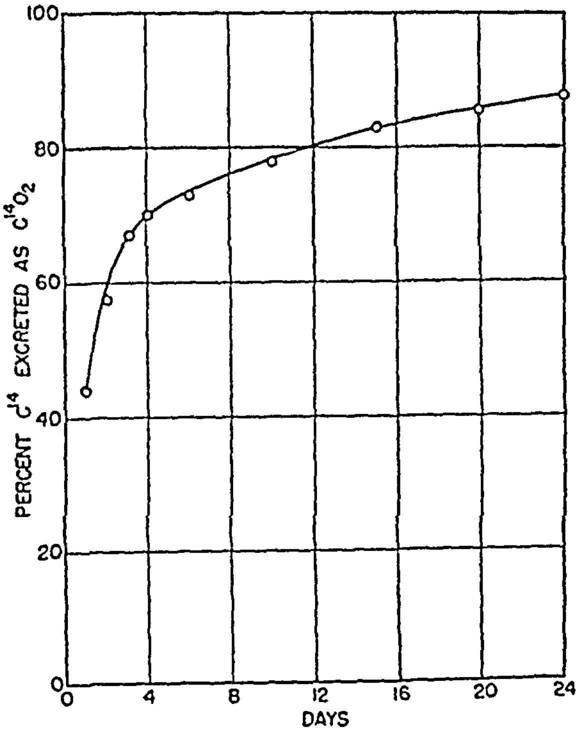

FIG. 4.
THE CUMULATIVE $C^{14}$ EXCRETION AS $C^{14}O_2$ IN PATIENT 1

The cumulative excretion after the 24th day is not shown, but by the 45th day it will amount to approximately 92 per cent of the injected dose.

## DISCUSSION

The cumulative excretion of $C^{14}$ is so rapid that in Patient 1 at the end of one day approximately 40 per cent has been eliminated by this route, and by the end of five days 60 per cent; by 20 days, approximately 83 per cent (see Figure 4). Furthermore, in all four patients by the end of 40 to 75 days there was no significant measurable activity in the breath; *i.e.*, less than 0.04 dis./

TABLE I

*The per cent of the administered dose excreted and half time for each of the three rate processes*

| Patient | $T_1$ (1/2) | Per cent | $T_2$ (1/2) | Per cent | $T_3$ (1/2) | Per cent | Total per cent excreted |
|---|---|---|---|---|---|---|---|
| | *hours* | | *hours* | | *hours* | | |
| 1 | 3 | 36 | 31.2 | 27 | 223 | 29 | 92 |
| 2 | 2.7 | 32 | 40 | 13 | 163 | 38 | 83 |
| 3 | 2.5 | 13 | 36 | 21 | 336 | 52 | 86 |
| 4 | 3.3 | 22 | 16 | 17 | 240 | 49 | 88 |

min./mg. $BaCO_3$. It is probable that there is a slower component, the radioactivity of which could be detected by more sensitive methods (10, 11); however, this could represent only a small fraction of the administered dose.

The relation of these three different rate processes to known biochemical processes is not known. However, the measurement of the uptake of $C^{14}$ in the plasma proteins and their turnover time suggests that the slowest component may represent the rate constant for protein synthesis and turnover (12).

The finding that the slowest measured rate of excretion is of the same order of magnitude in man as in mice should permit the extrapolation to man of animal investigations with similar $C^{14}$ labeled compounds. This may allow the use of such animal data as the basis for the determination of the hazard of using $C^{14}$ in clinical investigation. In addition to the 83 to 92 per cent excreted via the lungs, some 5 per cent of the injected dose is excreted in the urine during the first 14 days, and a negligible amount in the feces (12). An additional 2 per cent is present in the red blood cells as hemoglobin and will not be eliminated until the red cells are destroyed at the end of approximately 120 days in normals (8).

Analysis of $C^{14}$ content of the tissues obtained at autopsy from Patient 3 shows that approximately 10 per cent of the injected activity was still present in the tissues 106 days after administration of the labeled glycine. If this remaining activity is eliminated from the body by the lungs by a single first order rate process, the elimination half time for this component can be estimated by use of the equation $N = 1.44\ N_0\ T\frac{1}{2}$ where N ($2.2 \times 10^7$ dis./min.) is equal to the total $C^{14}$ to be excreted by this rate process and $N_0$ is the zero time extrapolated value of the component. $N_0$ can be estimated to be no greater than 120 dis./min. (0.05 dis./min./mg. $BaCO_3$) ($2.4 \times 10^3$ mg. $BaCO_3$/min. breathing) since the effect of this component cannot be seen in the graph in Figure 3, which is typical of the four patients.

As calculated in this manner, the half time is approximately 90 days. This represents the minimum value that this component could take since what is probably the maximum value for the zero time extrapolated value of this component has been chosen.

Sprinson and Rittenberg (13) have calculated that there is a protein turnover component in man with a half time of 158 days. The data presented here suggest that there is a $C^{14}$ turnover component associated with labeled glycine administration of at least 90 days, which is in fair coincidence with that of Sprinson and Rittenberg, considering the assumptions made.

Since approximately 90 per cent of the administered $C^{14}$ is rapidly eliminated by processes which can at the present time be measured directly, the radiation dose from the small fraction which is retained for a longer period is negligible at the present administered dosage level of 100 microcuries, and thus there is no hazard involved in this use of $C^{14}$. Finally, it should be pointed out that even if there were no elimination of the administered $C^{14}$, 100 microcuries can still be considered a safe tracer dose since it will deliver on the average less than 0.006 r per day of radiation to the tissues.

## SUMMARY AND CONCLUSIONS

1. The rate of excretion of $C^{14}$ via the lungs when administered as Glycine-2-$C^{14}$ in three patients has been presented.

2. This pulmonary excretion has been described in terms of three rate processes, the slowest of which has a half time of seven to 14 days.

3. In 55 days approximately 83 to 92 per cent of the $C^{14}$ is excreted via the lungs, 5 per cent via the urine, and 2 per cent is retained in the red blood cells during their life span.

4. Autopsy data in Patient 3 suggests that there is at least a fourth component of breath excretion with a half time of 90 days or greater.

5. The lack of hazard involved in the use of $C^{14}$ in the manner described is discussed from the standpoints of rapid excretion of 90 per cent of the dose and retention of approximately 10 per cent in chemical compounds having a longer turnover time.

## ACKNOWLEDGMENT

The authors wish to thank Doctors Hardin B. Jones and John C. Weaver for their help in carrying out these studies. They also wish to thank J. Krone, M. Lazarus, H. Lee and Mr. Ikeda for their technical assistance.

## ADDENDUM

*Patient 1 (W. F.)* is a 29 year old white male who has had chronic lymphatic leukemia of three years duration and who at the time of these studies was in almost complete clinical remission.

*Patient 2* (*H. M.*) is a 52 year old white male with polycythemia vera of 12 years duration. At the time of these studies he was in hematological remission, but shortly thereafter began to relapse hematologically.

*Patient 3* (*G. J.*) was a 52 year old white female with chronic myelogenous leukemia of one year's duration. At the time of these studies the white blood count was in the 300,000 range, the platelet count was 600,000 and the red blood count 3.0 and the hemoglobin 6.9 grams. This patient pursued, despite treatment, a down hill course and expired in August 1950.

*Patient 4* (*M. D.*) is a 50 year old white female with polycythemia vera of 14 years duration. At the time of these studies the red blood count was 6.5, hemoglobin 15 grams, white blood count 6,500, platelets 280,000. The patient has maintained this hematological picture until the present time.

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# EFFECT OF PLATELET EXTRACT ON HEMOPHILIC BLOOD

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(Submitted for publication July 1, 1950; accepted, October 23, 1950)

The function of platelets in the clotting process is still a matter of controversy. In Morawitz's classic formulation (1) the role of furnishing thromboplastin was attributed to them. Brinkhous, who agrees with this concept, has demonstrated that platelet-free, "quasi-hemophilic" plasma does not clot (2). According to him (2, 3) the thromboplastin contained in platelets is set free during clotting through the action of a plasma factor functioning as a thrombocytolysin. The fundamental defect in hemophilia is thought to be a diminution or absence of this plasma factor. Other investigators (4, 5), however, have maintained that the quantities of thromboplastin found in platelets are too small to be of significance in coagulation. According to Quick (3, 6) they liberate an enzyme, thromboplastinogenase, which interacts with the plasma factor, thromboplastinogen, to form thromboplastin. Hemophilia again is interpreted to be a deficiency of the plasma factor, *i.e.*, a lack of substrate for the platelet enzyme. Ware and associates (7) analyzed platelet extracts and found traces of thromboplastin, an accelerator analogous to serum Ac-globulin and a factor which enhances the action of thrombin on fibrinogen.

During the study of two patients with hemophilia-like disease caused by circulating anticoagulants which seemed to inhibit either the formation or utilization of thromboplastin, we found (8) that a concentrated homogenate[3] of ground-up platelets was able to restore the markedly prolonged clotting times to normal. Quick and Stefanini (9) have suggested that in hemophilia-like disease the anticoagulant may inhibit the action of the platelet enzyme on the plasma thromboplastin precursor, and the acceleration of clotting observed by us seemed to be attributable to an overcoming or neutralization of the anti-platelet enzyme factor by the extract. However, we observed a similar shortening of the coagulation time when such a concentrated platelet preparation was added to hemophilic blood (8), which is deficient in plasma factor *but has normal platelets.* It was established that these extracts had definite thromboplastic activity, and the problem presented itself whether this could account for the noted effects. It was decided, therefore, to investigate and compare the action of platelet homogenates and dilutions of tissue thromboplastin on the clotting time and prothrombin consumption time of a group of patients with hemophilia.

## METHODS

### 1) *Preparation of Platelet Extract.*

A modification of the procedure described by Mann and colleagues (10) was employed. Citrated blood (one part of 1.34% sodium citrate to four parts of whole blood) is collected with an ordinary syringe and centrifuged soon after collection at 700–800 RPM for one half hour. The supernatant platelet-rich plasma is respun at the same speed for 15 minutes, removed from the few remaining red cells, and again centrifuged in Wassermann tubes at 3,000 RPM for 30 minutes.

The platelets, which still may be contaminated with a few red and white cells, are found to form a sticky pellet at the bottom of the tubes. The supernatant plasma is poured off and measured; the pellet is dislodged and broken up with a wooden applicator and shaken vigorously with a saline-citrate solution (0.1 cc. of sodium citrate to 10 cc. of normal saline). The platelets are then sedimented again by centrifuging at 3,000 RPM for 10 minutes. This process of washing is repeated three more times. After the final washing the platelets are suspended in saline-citrate and triturated in a mortar for two to three minutes. The final volume of this suspension of ground-up platelets in saline-citrate is adjusted so that 1 cc. of platelet homogenate corresponds to 10 cc. of original plasma, care being taken to correct for the 20% dilution of the plasma with citrate. The platelet extract is then ready for use but can be kept in the frozen state for 24 hours without loss of potency.

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[1] Ruth Reader Fellow in Hematology.

[2] The Department is in part supported by the Michael Reese Research Foundation and the Hematology Research Foundation. Acknowledgment is also made for support of these studies to the Hulda B. and Maurice L. Rothschild Foundation for Scientific Research.

[3] For the sake of convenience the terms platelet "extract" and "homogenate" will be used interchangeably.

*2) Prothrombin Time.*

Determinations were made with the one-stage method of Quick, using a commercial preparation of thromboplastin (Maltine). Normal values range from 11 to 14 seconds.

*3) Clotting Time.*

The Lee-White clotting time was done always in three test tubes (8 × 75 mm.) at room temperature. Values up to 20 minutes are considered to be normal (11). When the effect of various substances on the clotting time was tested, the material was placed first into each tube, 1 cc. of blood then added, the tube inverted once, and the clotting time measured in the usual manner.

*4) Prothrombin Consumption Time.*

This test was performed by a slight modification of Quick's (4) method. About 5 cc. of blood were drawn into an ordinary syringe with a silicone-coated needle by clean venipuncture and discarded. Then a blood sample for the test was collected in an iced, siliconized syringe through the same needle and centrifuged in iced, siliconized tubes at 1,700 RPM for 10 minutes. The plasma was tranferred in 1 cc. portions to uncoated tubes containing the substance whose effect on prothrombin consumption time was to be tested and allowed to clot at 37°C. One hour after coagulation the clot was rimmed and the tube centrifuged at 4,500 RPM for one minute. The prothrombin consumption time was determined immediately using freshly prepared $BaSO_4$-plasma as a source of fibrinogen. Normal values, in our hands, are above 17 seconds, signifying that more than 60% of the prothrombin activity has disappeared. Further determinations of prothrombin consumption time were made on the serum, which had been allowed to remain in contact with the clot at 37°C, at varying intervals up to one and one half hours. There is normally a progressive lengthening of the prothrombin consumption time within this period.

*5) Preparation of Thromboplastin Dilutions.*

Thromboplastin (Maltine) was prepared as for the routine prothrombin time determination. The final thromboplastin solution was tested on normal oxalated plasma. When found to be satisfactory (prothrombin time of 11 to 14 seconds), the thromboplastin solution was diluted with normal saline until the desired concentrations were obtained.

## RESULTS

### *1) Determination of the Thromboplastic Activity of Platelet Extracts.*

The one-stage prothrombin time procedure of Quick was used as an index of the thromboplastic activity of platelet homogenates. 0.1 cc. of platelet extract and of increasing dilutions of thromboplastin were substituted for undiluted thromboplastin, and the "prothrombin times" determined on normal, oxalated plasmas in the usual manner. This comparison was performed separately for each platelet preparation, and their average thromboplastic activity was found to be equivalent to that of a thromboplastin dilution of approximately 1:800. Table I gives typical values obtained in such experiments with four different platelet extracts.

### *2) Effect of Platelet Extracts on Clotting Time.*

Platelet extracts always were effective in shortening the clotting times of the bloods of eight hemophiliacs (Table II). Although the amount of extract used in these experiments was usually 0.3 cc., as little as 0.05 cc. was sufficient to bring the clotting times into the normal range in almost all instances. This effect cannot be attributed to contamination with either plasma or red cells, even though it has been shown that quantities of normal plasma as small as 0.001 cc., when added to 2 cc. of hemophilic blood, increase the rate of clotting considerably (12). When the clot-promoting ability of equal quantities of dilutions of fresh oxalated plasma, triturated washed red cells, and platelet extracts was compared, significant shortening of the clotting occurred only with the diluted plasma

TABLE I

*Comparison of thromboplastic activity of platelet extracts and dilutions of thromboplastin on normal plasmas*

| Source of thromboplastin | "Prothrombin" time in seconds | | | |
|---|---|---|---|---|
| | Subject A | Subject B | Subject C | Subject D |
| Thromboplastin (Maltine) undiluted | 12 | 13½ | 12 | 14 |
| Thromboplastin (Maltine) 1: 500 | 63 | 64 | 61 | 66 |
| Thromboplastin (Maltine) 1: 800 | 75 | 72 | — | 79 |
| Thromboplastin (Maltine) 1:1,000 | 80 | 81 | 71 | 94 |
| Thromboplastin (Maltine) 1:2,000 | 93 | 88 | 85 | 93 |
| Platelet extracts | 74 | 66 | 74 | 90 |

TABLE II

*Effect of platelet extracts and of thromboplastin dilutions on clotting times of hemophilic blood*

| Patient no. | Effect of platelet extract (clotting times in minutes) |  | Effect of thromboplastin dilutions (clotting times in minutes) |  |  |  |  |
|---|---|---|---|---|---|---|---|
|  |  |  |  | Thromboplastin dilutions |  |  |  |
|  | Normal saline 0.3 cc. | Platelet extr. 0.3 cc. | Normal saline 0.3 cc. | 1:500 0.3 cc. | 1:800 0.3 cc. | 1:1,000 0.3 cc. | 1:2,000 0.3 cc. |
| 1 | 26½ | 9 | 23 | 8 | 8 | 8 | 11 |
| 2 | 33 | 9 | 35 | 8 | — | 9 | 13 |
| 3 | 35 | 15 | 35 | 9 | 15 | 24 | 25 |
| 4 | 44 | 14 | 22 | 8 | — | 9 | 10 |
| 5 | over 150 | 8 | — | — | — | — | — |
| 6 | 130 | 13 | 130 | 7 | — | 8 | 34 |
| 7 | 69 | 17 | 69 | 8 | 11 | 21 | 21 |
| 8 | 120 | 16 | 120 | 6 | 8 | 9 | — |

and the platelet extracts. The potency of the platelet preparations was essentially unaffected by repeated washings prior to trituration, demonstrating that contamination with antihemophilic globulin was not involved (Table III).

### 3) *Effect of Thromboplastin Dilutions on Clotting Time.*

Saline thromboplastin dilutions of 1:500 and 1:800 reduced the clotting times to normal values in all instances (Table II). Higher dilutions (1:1,000, 1:2,000) were less effective in some patients but always caused a significant shortening of the clotting time. A rough quantitative relationship seems to exist between the rate of clotting and the amount of thromboplastin added.

### 4) *Effect of Platelet Extracts on Prothrombin Consumption Time.*

The prothrombin consumption time was determined on 27 separate occasions in our eight patients. In five patients the values obtained never were higher than 13 seconds, whereas in three (cases 1, 3, and 4, Table IV) the prothrombin consumption time showed definite, spontaneous fluctuations, sometimes even reaching the normal range. In two instances we observed a normal prothrombin consumption time in the presence of a prolonged clotting time. The effect of platelet extracts was tested on 12 separate occasions (Table IV). A change to normal values—above 17 seconds—was obtained six times (twice with 0.2 cc., and four times with 0.4 cc.), whereas in five experiments the prothrombin consumption time was raised only slightly. The ability of equal amounts of platelet extract to produce an increased prothrombin consumption time varied in one patient (case 6) on different days. This cannot be blamed on differences in potency of the homogenates used, since extracts were found to be effective in one patient, but ineffective in another when tested simultaneously. Therefore, it seems

TABLE III

*Comparison of the effect of platelet extract, triturated red cells, and diluted normal plasma on the clotting time of hemophilic blood*

| Material added (0.3 cc.) to 1 cc. of hemophilic blood | Clotting time in minutes |  |  |  |
|---|---|---|---|---|
|  | Subject A | Subject B | Subject C | Subject D |
| None | 34 | — | — | — |
| Normal saline | 33 | 88 | 95 | 22½ |
| Platelet extract, washed 3× | 9 | 18 | 14 | 12 |
| Platelet extract, washed 5× | — | 18 | 17 | 13½ |
| Platelet extract, washed 9× | — | 21 | 25 | 12½ |
| Triturated, washed red cells (1:10) | 25 | — | 83 | — |
| Diluted plasma (1:10) | 24 | 16 | 14 | — |
| Diluted plasma (1:100) | — | 29 | 22 | — |

TABLE IV

*Prothrombin consumption time one hour after clotting with addition of various materials to 1 cc. of "native" plasma*

| Patient no. | Prothrombin time in seconds | Prothrombin consumption time in seconds | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Nothing added | Normal saline 0.2 cc. | Platelet extract | | 0.2 cc. Thromboplastin dilutions added | | | | |
| | | | | 0.2 cc. | 0.4 cc. | 1:200 | 1:500 | 1:800 | 1:1,000 | 1:2,000 |
| 1 | 12½ | 15 | — | — | — | — | — | — | — | — |
| | 12 | 17 | — | — | — | — | — | — | — | — |
| | 12½ | 13 | — | — | — | — | — | — | — | — |
| | — | 11½ | 13 | 18 | — | — | 14½ | 13½ | 13½ | 13 |
| 2 | 13 | 11½ | — | — | — | — | — | — | — | — |
| | 14 | 13 | — | — | — | — | — | — | — | — |
| | — | 12 | — | — | — | — | — | — | — | — |
| | — | 12 | 12½ | — | 17 | — | 17½* | — | 13* | — |
| 3 | 13 | 22 | — | — | — | — | — | — | — | — |
| | 14 | 22½ | — | — | — | — | — | — | — | — |
| | 12½ | 11½ | — | — | 17½ | — | — | — | — | — |
| | — | 16 | — | — | — | — | — | — | — | — |
| | 13 | 13 | — | — | — | — | — | — | — | — |
| | 13 | 19† | — | — | — | — | — | — | — | — |
| 4 | 11½ | 11½ | — | — | — | — | — | — | — | — |
| | 11½ | 12 | — | — | 17 | — | — | — | — | — |
| | — | 18‡ | — | — | — | — | — | — | — | — |
| 5 | 11 | 19½ | — | — | — | — | — | — | — | — |
| | 13 | 11 | 11½ | 15½ | 17 | 18 | 17 | 18½ | 16 | — |
| | — | 10 | — | — | — | — | — | — | — | — |
| | — | 12½ | — | — | — | — | — | — | — | — |
| 6 | 11½ | 10½ | 10½ | 23½ | — | — | — | — | — | — |
| | — | 10½ | — | 14 | — | — | 12 | — | 11 | 10½ |
| | 11½ | 9½ | 9½ | 13½ | 14½ | 14 | 11 | 10½ | 11 | — |
| 7 | 13½ | no clot | 9 | 13½ | 15 | — | 10½ | 10 | 10½ | 11 |
| | — | no clot | — | 16 | — | — | — | — | — | — |
| 8 | 14 | no clot | 11 | 14½ | 15½ | — | 12 | 15½ | 15½ | 11½ |
| | 13½ | 10 | 10 | 15 | 16 | 23 | 20½ | 10½ | — | — |

* Only 0.5 cc. of plasma used
† Clotting time 35 minutes
‡ Clotting time 22 minutes

likely that the irregular responses are determined by variables present in the plasmas.

### 5) *Effect of Thromboplastin Dilutions on the Prothrombin Consumption Time.*

Thromboplastin in dilutions of 1:1,000 or 1:2,000, added to hemophilic plasma, had little effect. Larger concentrations (1:200 to 1:800), however, were capable of producing a normal prothrombin consumption time at times. No parallelism appeared to exist between the ability of dilute solutions of thromboplastin to shorten the coagulation time and to increase the prothrombin consumption time. A quantity of thromboplastin, which clots hemophilic blood with normal speed, may be unable to affect the prothrombin consumption time in the same patient. These results are to be expected, since it is known that the prothrombin consumption time of hemophilic blood—which has clotted because of contamination with tissue thromboplastin during venipuncture—may be pathologic in the presence of a normal clotting time (13). Again it is to be noted that individual patients showed marked differences in the extent to which thromboplastin dilutions could alter their prothrombin consumption time.

## DISCUSSION

Our experiments showed that concentrated aqueous homogenates of human platelets consistently reduced the clotting times to normal in eight hemophilic subjects. The effect on the prothrombin consumption time, however, was not always significant. Platelet extract was found to possess a thromboplastic activity equivalent to that of a dilution of lung thromboplastin of about 1:800 as measured with the one-stage "prothrombin time" technique. Thromboplastin solutions of this potency exerted approximately the same effect on the clotting and prothrombin consumption times as the platelet preparations. These findings seem to indicate that the platelet extracts most likely acted by virtue of their thromboplastic activity. We choose this term since the nature of "thromboplastin" is not clearly defined at present. Many materials exhibit thromboplastic activity, but the responsible agents are not necessarily identical. At least two thromboplastins with different chemical properties have been found in lung and brain tissues, respectively (14).

Previous investigations have demonstrated that platelets contain traces of thromboplastin (4, 5, 7, 15), an accelerator analogous to serum Ac-globulin (7, 15), and a factor enhancing the action of thrombin on fibrinogen (7). It is unlikely that the platelet accelerator accounts for the observed results, since it has been shown that purified Ac-globulin does not influence significantly the clotting time of hemophilic blood (16). A potentiation of the action of thrombin on fibrinogen does not explain the increased prothrombin consumption time which was observed on several occasions and, therefore, cannot be the primary mechanism by which the platelet homogenate exerts its effect.

Other clotting factors also can be ruled out. Thrombin is not present in the platelet homogenates since they do not clot $BaSO_4$-plasma (17). No serum prothrombin conversion accelerator has been found in platelet extracts (18). Plasma antihemophilic globulin is removed by repeated washings during the preparation of the platelet extracts. It does not appear reasonable that thromboplastinogenase, the platelet enzyme specifically postulated to interact with the plasma factor thromboplastinogen (antihemophilic globulin), should have a direct activating effect on prothrombin. This would have to be assumed since no, or very little, thromboplastinogen can be considered to be present in hemophilic blood. By exclusion, therefore, it appears that platelet extract acted by virtue of its thromboplastic activity. Our comparative studies with dilutions of tissue thromboplastin, while not furnishing direct evidence, also strengthen this interpretation by demonstrating that only very small amounts of thromboplastin are fully capable of producing all the effects noticed with platelet extract.

Most investigators agree that interaction of both platelet and plasma factors is necessary to form an active thromboplastin for normal clotting. Conley and co-workers (19) have shown that "complete" clotting—as measured by normal clotting and normal prothrombin consumption times—takes place only when both factors are present. In hemophilia the plasma factor is absent or decreased, while the platelets now are considered to be normal (2–4); in severe thrombocytopenia the picture is reversed (20). In both conditions the prothrombin consumption time is usually abnormal; in hemophilia the clotting time is increased, but in thrombocytopenia it is within normal limits. In the latter disorder this finding is ascribed to either release of sufficient thromboplastinogenase by the few remaining platelets (3, 4) or direct activation of the plasma thromboplastin precursor by glass surfaces (21). In our experiments platelet extracts on several occasions produced both normal clotting and prothrombin consumption times in hemophilic subjects in whom the plasma factor was absent or markedly decreased. Since the extract also was free of this latter factor, it must have provided in these instances the end product of the interaction of platelet and plasma factors, *i.e.* thromboplastic activity.

Patek and Stetson (22), on adding a suspension of platelets of the same concentration as that found in normal plasma, observed no effect on the clotting time of hemophilic blood. This apparent discrepancy with our data may be explained by considering that the platelet preparations used in our experiments were *1)* triturated and *2)* 200 times more concentrated than theirs. It does not seem permissible to apply directly our interpretation of the effect of the extracts to the function of platelets *in vivo.*

Most of our data show a lack of parallelism between the changes in the clotting and the prothrombin consumption times, regardless of whether tissue thromboplastin or platelet homogenate was the agent studied. In other words, while either reagent uniformly accelerated clotting, the "consumption of prothrombin" often was not increased significantly. Our data also suggest that patients with hemophilia show definite fluctuations in their clotting status. This is indicated by spontaneous variations in their clotting and prothrombin consumption times when tested repeatedly over prolonged periods in the absence of hemorrhage or treatment with transfusions. It is well known that hemophiliacs may exhibit periods of quiescence of bleeding manifestations to be followed by intervals marked by hemorrhages of varying severity (23). If it is assumed that these clinical and laboratory observations reflect differences in the patient's ability to form thromboplastin, this mechanism can be invoked to offer an explanation for the apparent inconsistencies of our observations. Both platelet extract and dilutions of thromboplastin supply thromboplastin in addition to that produced by the patient himself. It depends on the patient's own thromboplastin production—which does not seem to be constant—whether this extraneously furnished thromboplastin is sufficient to give a normal "consumption of prothrombin." Likewise, the occasional normal values of the prothrombin consumption time, which have been observed also by others (24), could be a reflection of a temporary improvement in the patient's clotting status. It is to be emphasized, therefore, that a normal prothrombin consumption time, even in the absence of therapy, does not exclude the diagnosis of hemophilia.

Considerable difference of opinion exists about what actually is being measured when the "prothrombin consumption time" is determined. It has been stated (18, 25) that during coagulation besides the disappearance of prothrombin, which lengthens the prothrombin time of the serum, accelerator factor(s) evolve, which shorten it. The release of adsorbed thrombin from the fibrin meshwork also may influence this value (26). However, even when these mechanisms are taken into consideration we are at a loss to explain our two observations of a normal prothrombin consumption time in the presence of a prolonged coagulation time.

## SUMMARY

1. Addition of a concentrated homogenate of ground-up platelets consistently reduces the clotting times of hemophilic bloods to normal. The prothrombin consumption time is increased regularly but not always significantly.

2. These effects can be explained most satisfactorily by attributing them to the thromboplastic activity of the platelet preparations.

3. When tested repeatedly over prolonged periods hemophiliacs, in the absence of therapy, may occasionally show a normal prothrombin consumption time. It is postulated that the fluctuations in the clotting status may reflect variations in the patient's ability to produce thromboplastin.

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# PEPTIDASE ACTIVITY IN LEUCOCYTES, ERYTHROCYTES AND PLASMA OF YOUNG ADULT AND SENILE SUBJECTS[1, 2]

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(Submitted for publication July 10, 1950; accepted, October 23, 1950)

## INTRODUCTION AND REVIEW OF THE LITERATURE

In the present work an attempt was made to find out whether the peptidase activity of the human blood changes with age. Preliminary to this a study of the characteristics of peptide hydrolysis was undertaken, and the degree of enzymatic activity in the various blood constituents was investigated.

Grassmann and Heyde (1) demonstrated the ability of serum to hydrolyse di- and polypeptides. Various authors have found that the peptidase activity of serum is increased in certain pathological conditions, such as during inflammatory processes, after bone fracture, and after burns (1–4). Concerning the possible source of the serum peptidases Zamecnik, Stephenson, and Cope (4) suggest that they may derive from muscle, skin, subcutaneous tissue and erythrocytes. Fruton and co-workers (5, 6) believe that peptidases may have a common origin, most probably the lymphoid cells present in the various tissues, and that the peptidase activity of serum might be due to the disintegration of lymphoid tissue.

Peptidase activity in erythrocytes has been demonstrated by a number of investigators (4, 7–10). Johansen and Thygesen (8) showed that per unit volume the rate of hydrolysis of alanylglycylglycine (AGG) by human erythrocytes is 40–50 times higher than by serum. Merten and Winschuh (11) report an increase in di-peptidase activity of human erythrocytes in various pathological conditions.

It has long been known that leucocytes are able to break down peptides [compare the literature review of Husfeldt (10); Merten and Winschuh (11)]. Husfeldt (10) demonstrated di-, tri-, and tetra-peptide hydrolysis by leucocytes from a normal subject and a patient with myeloid leukemia. Hydrolysis of alanylglycine by leucocytes from patients with lymphatic leukemia has been observed by Oelkers (12).

Little is known about the relation of the age of the organism to the proteolytic activity of blood. Goldenberg and Kondrachina (13) reported that the rate of autolysis of rabbit serum at pH 1 decreases with the age of the animal. Maver and co-workers (14), on the other hand, noted that serum from old normal rats apparently hydrolysed dl-leucylglycine at a faster rate than did the serum from younger rats.

## SUBJECTS

Two groups of subjects were studied. The first group included 15 members of the staff, nine male and six female, ranging in age from 19 to 32 years. The second group of 12 comprised a number of senile patients, five male and seven female, in a mental institute. The members of this group were not bedridden, and none of them suffered from any known organic or infectious disease. Their ages ranged from 67 to 90 years.

## METHOD

Twenty ml. of non-fasting blood was obtained from the cubital vein in the morning, and was poured immediately into flasks containing 40 mg. of potassium oxalate. A white blood count and differential count were made immediately. The white blood cells were separated by centrifugation of whole blood in waisted tubes (15). Plasma was removed by means of a capillary pipette. The buffy layer which contained white blood cells and platelets, plus a few red blood cells, was transferred with a second capillary pipette to a centrifuge tube, suspended in about 2 ml. of physiological saline solution and centrifuged at low speed for approximately two minutes. (Longer centrifugation caused the cells to adhere together in small clumps which could not be broken up on shaking.) After centrifugation the clear supernatant was sucked off with a pipette and the cells resuspended in from 2 to 4 ml. of saline and stored overnight in the refrigerator. The red blood cells were pipetted with a long capillary pipette from the bulb of the centrifuge tube and washed in the same manner as the white cells. The concentration of cells in these saline suspensions was determined by means of the standard

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[1] This research was aided by a grant from the National Research Council of Canada.

[2] An abstract of this paper appeared in Proceedings of the Canadian Physiological Society, October, 1950.

clinical procedures, using Turck's and Hayem's dilution fluids. All counts were made in quadruplicate.

The substrate used was glycylglycylglycine (GGG). Hydrolysis was measured by the micro-titration technique of Grassmann and Heyde. The reaction mixture was set up in triplicate in glass-stoppered 2 ml. volumetric flasks. It was composed of 1 ml. of 0.1 M substrate, 0.2 ml. of 0.01 M cobalt sulfate, 0.01 ml. toluene and 0.8 ml. of plasma, or 0.6 ml. of distilled water and 0.2 ml. of cell suspension containing approximately $10^6$ leucocytes or $10^8$ erythrocytes.[3] The reaction was carried out in a water bath at a temperature of 39°C and at a pH of 7.0, maintained within less than 0.1 unit by the buffering effect of the substrate which had been brought to pH 7.0 with 0.5 N NaOH. Hydrolysis during a two to four hour period was measured at frequent intervals by titrating 0.2 ml. samples with 0.01 N KOH (final concentration 90% alcohol), using thymolphthalein as indicator. At the dilution of red blood cells used no serious difficulty with the endpoint was encountered.

## RESULTS

Preliminary experiments regarding some of the characteristics of the hydrolysis of GGG will be described first. The rate of hydrolysis by plasma, white cells, or red cells, is constant until about 70% of the substrate is split, assuming complete splitting of one peptide linkage (see Figure 1). Beyond 100% theoretical splitting of a single bond of the tripeptide, hydrolysis continues but at a slower rate. To what extent the breakdown of the di-peptide contributes to the earlier stages of GGG hydrolysis is not known. The rate of hydrolysis by plasma, erythrocytes, and leucocytes was found

TABLE I

*The effect of dilution of blood components on the rate of hydrolysis of GGG*

Substrate 0.05 M GGG, pH 7.0

| Enzyme source | Concentration per ml. | Per cent hydrolysis per hour | |
|---|---|---|---|
| | | Observed | Calculated for 0.2 ml./ml. |
| Plasma* | 0.10 ml.<br>0.15 ml.<br>0.20 ml.<br>0.26 ml.<br>0.40 ml. | 7.0<br>9.5<br>12.0<br>17.0<br>24.4 | 14.0<br>12.7<br>12.0<br>12.6<br>12.2 |
| | | Observed | Calculated for 1 million cells/ml. |
| R.B.C. | 96 million cells<br>72 million cells<br>48 million cells | 45<br>34<br>23 | 0.48<br>0.47<br>0.48 |
| W.B.C. | 1.34 million cells<br>0.96 million cells | 16.0<br>12.7 | 11.2†<br>12.3† |

* The plasma was slightly hemolysed which accounts for the high rate of hydrolysis.

† Values corrected by subtraction of the rate of hydrolysis due to the presence of erythrocytes.

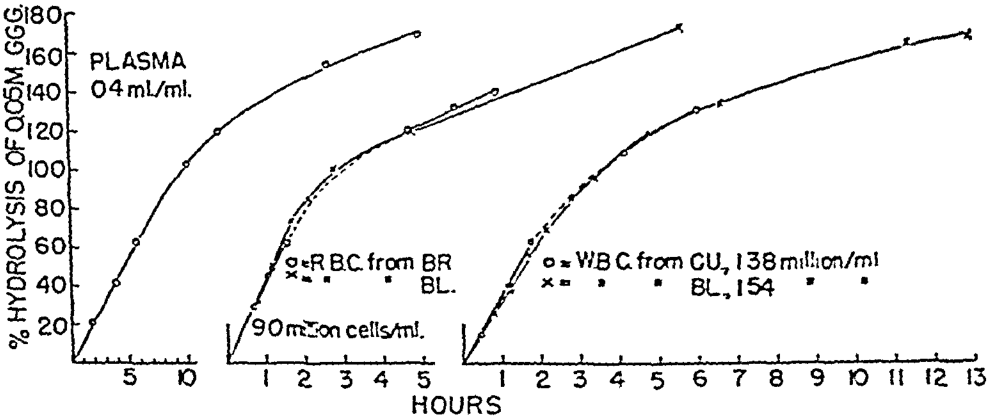

FIG. 1. THE COURSE OF HYDROLYSIS OF GGG BY PLASMA, LEUCOCYTES, AND ERYTHROCYTES

The reaction follows a similar pattern with all three enzyme sources, i.e., the hydrolysis rate is constant until about 70% of the substrate is split; after 100% theoretical splitting of one peptide bond of the tripeptide further hydrolysis occurs but at a slower rate.

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[3] In order to facilitate cytolysis the cell suspensions were incubated with the distilled water and the cobalt sulfate solution for 15 minutes before the addition of substrate, and the tubes vigorously agitated. Cell counts immediately after substrate addition (zero experimental time) and during the course of hydrolysis showed that the red cells were completely hemolyzed but only about 40% of the white cells from both young adult and senile subjects were cytolyzed at zero time and about 50% after two hours incubation. In all experiments the peptidase activity is based on the cell count before cytolysis occurred.

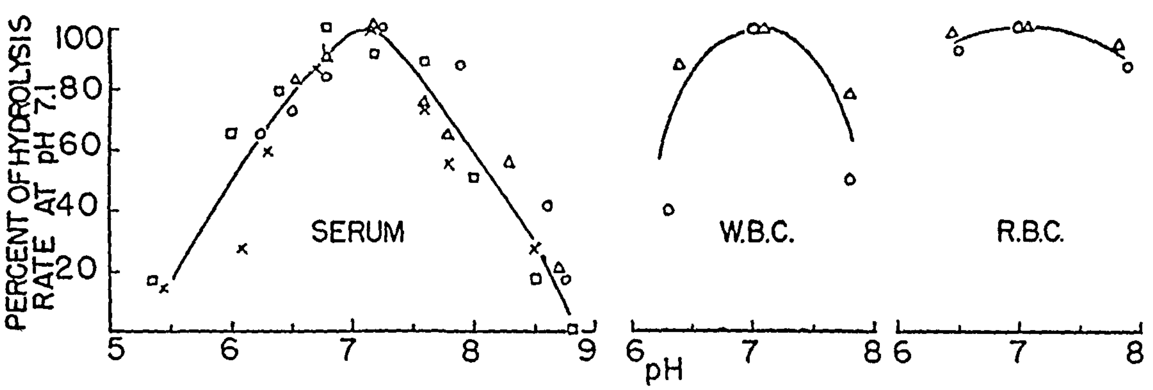

Fig. 2. Effect of pH on the Hydrolysis Rate of GGG by Serum, Leucocytes, and Erythrocytes

The substrate was adjusted to the desired pH by adding the required amounts of HCl or NaOH. The drift in pH during the course of hydrolysis was negligible. Similarly marked points in each curve were obtained on samples of the same blood.

to be directly proportional to the concentration of these constituents in the reaction mixture (see Table I). The effect of metal ions on the hydrolysis rate is shown in Table II. Cobalt activates, magnesium and manganese inhibit the hydrolysis by plasma, red cells and white cells. It may be noted that a similar effect of these metal ions has been observed in this laboratory with the hydrolysis of l-leucylglycylglycine (LGG) by human serum (2). The effect of pH on the hydrolysis of GGG by various blood constituents is shown in Figure 2. Optimal hydrolysis occurs around pH 7.1.

The similar characteristics of the hydrolysis of GGG makes it likely that the same enzyme mechanism is involved in plasma, leucocytes, and erythrocytes.

The effect of temperature was studied using serum as the source of the enzyme. The rate of hydrolysis increases with increasing temperature between 0° and 48°C, but at higher temperatures inactivation sets in (see Figure 3).

Regarding the relative activity of the blood components, it may be seen from Table III that in normal young people the average rate of hydrolysis per cell is about 40 times higher in white blood cells[4] than in erythrocytes. In view of the high concentration of erythrocytes in blood, however, the activity of a sample of whole blood is mainly due to the hydrolysis by red blood cells. Using the average rates of hydrolysis of GGG in young adults and average normal values for red and white cell counts and plasma volume (16) it may be estimated that in a unit of whole blood the activity of the white blood cells is about six times greater, that of the red blood cells about 100 times greater than that of plasma. If the activity

TABLE II

*Effect of metal ions on the hydrolysis of GGG by plasma, leucocytes, and erythrocytes*

Per cent hydrolysis per hour of 0.05 M GGG of pH 7.0, calculated for a concentration of 0.2 ml. plasma, or $10^6$ cells per ml. reaction mixture.

| | Plasma | R. B. C. | W. B. C. | |
|---|---|---|---|---|
| | | | Age of subject | |
| | | | 28 Years | 67 Years |
| No activator | 4.0 | 0.25 | 15 | 21 |
| Co (0.001 M) | 5.6 | 0.43 | 19 | 31 |
| Mn (0.001 M) | 3.1 | 0.16 | 10 | 16 |
| Mg (0.01 M) | 3.5 | 0.14 | 9 | 13 |

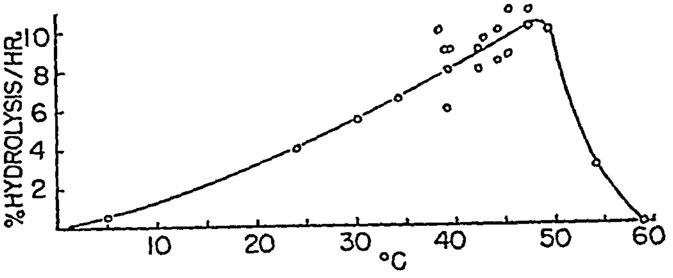

Fig. 3. Effect of Temperature on the Hydrolysis Rate of GGG by Serum

Values represent per cent hydrolysis by 0.2 ml. of serum per ml. reaction mixture. Serum was obtained at various times from one subject.

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[4] The hydrolysis of GGG by white blood cells has been corrected by subtraction of the rate of hydrolysis due to the presence of erythrocytes in the reaction mixture. In most cases this value was less than 10% of the total rate.

of one volume of plasma is compared with that of a volume of packed white and packed red cells, ratios of 1 to 480 to 125 are obtained. A similar proportion of enzyme activity among blood components was observed in experiments in which dl-leucylglycylglycine was used as substrate. The relative rates for plasma and red blood cells are somewhat higher, but of the same order of magnitude as the comparative values for AGG hydrolysis by human serum and erythrocytes estimated by Johansen and Thygesen (9). No clear cut data in the literature on the relative activity of white blood cells are known to us.

From Table III it may be seen that the rate of hydrolysis of GGG by white blood cells is considerably higher in the old age group than in the young adult group. The average values of hydrolysis of 0.05 M GGG per hour for one million cells per ml. of reaction mixture are 26 and 15% respectively. The difference is statistically significant ($P < 0.001$). (The averages for male and female subjects correspond closely in both groups.) When the values for plasma or red blood cells are compared no significant difference between the two groups is seen. No correlation between activity of white blood cells and the percentage of

TABLE III

*Hydrolysis of GGG by leucocytes, erythrocytes, and plasma of young and old people*

| Name | Age | Sex | Total W.B.C. | % Poly. | % Lym. | % Stab. | Hydrolysis % per hour* | | | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | $10^6$ WBC/ml. reaction mixture | $10^8$ RBC/ml. reaction mixture | 0.2 ml. plasma/ml. reaction mixture | |
| VE | 27 | M | | 17 | 47 | 26 | 18 | 0.35 | | |
| WO | 22 | M | | 26 | 67.5 | 0.5 | 17 | 0.27 | | |
| CH | 25 | M | | 56 | 42 | 0 | 14 | 0.27 | | |
| | | | 6,900 | 59 | 35 | 4 | 15 | 0.41 | 5.5 | |
| WI | 21 | F | | 69 | 25 | 0 | 14 | 0.32 | | |
| CA | 23 | M | | 61 | 33 | 0 | 18 | 0.25 | | |
| BR | 23 | M | 9,700 | 34.5 | 39 | 19 | 13 | 0.26 | | |
| | | | 8,800 | 50 | 41 | 6 | 12 | 0.34 | 7.0 | Repeat 2 weeks later |
| CU | 28 | F | 8,250 | 52 | 41 | 0 | 11 | 0.41 | | |
| | | | 11,675 | 51 | 39 | 4 | 20 | 0.36 | | 1st day menstruation |
| | | | 21,800 | 54 | 33 | 8 | 15 | 0.46 | | 2nd day menstruation |
| DY | 22 | F | 6,950 | 51 | 40 | 1 | 18 | 0.38 | | |
| PU | 22 | F | 5,950 | 57 | 34 | 5 | 16 | 0.39 | 6.2 | |
| KO | 26 | F | 7,500 | | | | 9 | 0.44 | 6.5 | |
| DE | 28 | F | | | | | 16 | 0.41 | | Jan. 6, 1950 |
| | | | | 65 | 27 | 6.5 | 19 | 0.43 | | April 4, 1950 |
| | | | | | | | 21 | 0.46 | | May 15, 1950 |
| HO | 26 | M | 7,100 | | | | 14 | 0.46 | | |
| MA | 32 | M | | | | | 20 | 0.42 | | |
| KA | 19 | M | 11,300 | 56 | 38 | 0 | 10 | 0.40 | 6.8 | |
| EL | 26 | M | 4,800 | | | | 16 | 0.47 | | |
| Average | | | | | | | 15 | 0.38 | 6.4 | |
| DA | 76 | F | | 70 | 14 | 13 | 28 | 0.33 | | |
| | | | 13,450 | 81 | 7 | 7.5 | 22 | 0.34 | | Repeat 13 days later |
| SM | 68 | F | | 47 | 48 | 0 | 18 | 0.31 | 7.5 | |
| MI | 82 | M | | | | | 26 | 0.45 | | |
| | | | 6,900 | 53 | 40 | 4 | 28 | 0.41 | 7.8 | |
| HA | 90 | M | 9,000 | 41 | 53 | 4 | 26 | 0.35 | 7.0 | |
| | | | 6,550 | | | | 26 | 0.31 | 7.2 | Repeat 2 months later |
| KY | 94 | F | 11,050 | 60 | 32 | 4 | 30 | 0.48 | 5.5 | |
| CA | 86 | M | 9,750 | 61 | 32 | 4 | 32 | 0.43 | [illegible] | |
| NO | 84 | M | 10,200 | 40 | 57 | 1 | 20 | 0.50 | 7.8 | |
| McG | 87 | F | 10,775 | 58 | 34 | 2 | 30 | 0.47 | | |
| HE | 89 | F | 10,525 | 59 | 38 | 2 | 33 | 0.38 | | |
| ME | 86 | F | 8,250 | | | | 26 | 0.24 | 6.2 | |
| BA | 67 | F | | | | | 25 | 0.40 | | |
| BR | 71 | M | | 42.5 | 48.5 | 8.5 | 23 | 0.35 | | |
| Average | | | | | | | 26 | 0.39 | 7.0 | |

* Per cent per hour of the hydrolysis expected on the complete splitting of one peptide linkage.

polymorphonuclear leucocytes and lymphocytes in the differential count was apparent, and the slight difference in the distribution of the cell types among the two groups could not account for the difference in enzymatic activity. The response to metal ion activation was compared in two subjects (Table II) and no significant difference was observed. The possibility that the difference in rate among the two groups is due to an increased state of metal activation inherent in the white blood cells of the old age group is therefore unlikely. Although no pH optimum for hydrolysis of white blood cells from old people was determined, it is evident that the differences in activity cannot be due to a different response to pH since all studies were carried out in the optimum pH range for the activity of white blood cells in young people.

## DISCUSSION

The increase in peptidase activity of white blood cells from aged subjects is noteworthy from a clinical standpoint in view of the role of leucocytes in processes of infection and tissue repair. It is possible, however, that the rise in peptidase activity might be a manifestation of increased protein metabolism in general, associated with old age. It must be pointed out that the old age group was composed largely of subjects who were institutionalized because of senile mental changes, and whose food intake was probably lower than that of the control group. The study will be continued on mentally normal old people. It is of interest that insulin, a polypeptide, has been found to lose its blood sugar reducing power when it is incubated with whole blood, and that blood from old people inactivates the hormone at a significantly higher rate than blood from young people (17). There was an indication that the leucocytes were responsible for this effect. This would be in line with the present observations, provided that a hydrolytic mechanism is involved in the inactivation of insulin.

The rise in peptidase activity of the white blood cells in the senile group was not accompanied by a significant rise in peptidase activity of plasma. This does not necessarily exclude the possibility that white blood cells are a source of plasma peptidase since the rate of dissolution of leucocytes rather than the actual activity of living cells may determine the level of enzymes in plasma. It has been observed that the leucocytes circulating in the blood stream of old people are older cells than those in the blood from young persons (18). This indicates that the rate of turnover is lower in old age. It should, however, be kept in mind that per unit of whole blood by far the greatest enzyme activity is found in the red blood cells. Whether erythrocytes, leucocytes, platelets, or other tissue cells are the main source of the enzyme in plasma can only be determined if the rate of breakdown and turnover, as well as the relative enzyme activity of these constituents, is known.

## SUMMARY

1. The hydrolysis of glycylglycylglycine (GGG) by the components of blood from young adult and from senile human subjects, and the effects of metal ions, pH, and temperature on the hydrolysis were studied.

2. Hydrolysis by plasma, erythrocytes and leucocytes was activated by cobalt, inhibited by magnesium and manganese and optimal at pH 7.1. In view of the similar characteristics of hydrolysis by the blood components it was concluded that the same enzyme mechanism must be involved in all cases.

3. The enzyme activity in the formed elements by far exceeded that of serum or plasma. In normal young people a volume of packed red cells was estimated to be over 100 times as active as a volume of plasma, and packed white blood cells nearly 500 times as active as plasma. Per unit cell the activity of leucocytes from young people was found to be about 40 times greater than that of the erythrocytes.

4. The rate of hydrolysis of GGG by leucocytes was found to be significantly higher in senile people than in young people, the average increase in rate being about 70%. On the other hand, no significant difference in rate of hydrolysis by plasma or erythrocytes of the two groups was observed.

5. The significance of these findings is discussed.

The authors are indebted to Miss Marylee Putnam for technical assistance.

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# THE EFFECT OF BILATERAL STELLECTOMY UPON THE CEREBRAL CIRCULATION OF MAN[1]

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(Submitted for publication July 13, 1950; accepted, October 23, 1950)

The role of the sympathetic nervous system in the intrinsic control of the cerebral circulation has always been controversial. Experiments heretofore have centered on the effects of stimulation of the cervical sympathetic nerves on changes in cerebral blood flow detected by instruments applied directly to the brain of animals. The results have generally agreed that cervical sympathetic stimulation causes only mild cerebral vasoconstriction. The marked effects of chemical agents ($CO_2$ in particular) in affecting the cerebral circulation have led to the probably correct conclusion that the intrinsic control of the cerebral circulation in health is predominantly mediated via chemical agents in the blood and that neurogenic vasomotor control is at best only weakly effective (1). However, recent clinical reports (2–5), which we can confirm from personal experience, on the efficacy of temporary blockade of the stellate ganglia in ameliorating symptoms of cerebral embolism and thrombosis, presumably by relieving collateral vasospasm, have increased interest in the role of the sympathetic system in the intrinsic control of the disturbed cerebral circulation.

Harmel and associates (6) studied the effects of bilateral stellate ganglion block with procain on the cerebral circulation of 13 unanesthetized normotensive and hypertensive patients and failed to find any effect whatsoever. They utilized the recently introduced nitrous oxide technique for determination of the cerebral blood flow (7) in their studies. Scheinberg (8) has recently confirmed their work.

We have had the opportunity to study the cerebral circulation of seven patients before and after bilateral stellate ganglionectomy and our findings are the subject of this report.

## METHODS

Patients selected for study were necessarily suffering from abnormalities for which it was hoped bilateral stellate ganglionectomy would be of benefit. Four patients (L. S., M. M., J. W. and W. N.) had postencephalitic Parkinson's disease; one (B. D.) was clinically diagnosed as advanced cerebral arteriosclerosis; one (R. H.) had hypertension and advanced cerebral atrophy (air-encephalogram); and one (A. A.) suffered with an old hemiplegia as a complication of essential hypertension.

Cerebral blood flow (CBF) was determined by the nitrous oxide technique (7). Mean arterial blood pressure (MABP) was measured from the femoral artery by a damped mercury manometer attached directly to a needle in the artery. Blood oxygen and carbon dioxide analyses were carried out by the manometric technique of Van Slyke and Neill (9). Blood pH measurements were made anaerobically at 37° C. using a glass electrode. Carbon dioxide tension in the blood ($pCO_2$) was calculated from the nomograms of Peters and Van Slyke (9). Cerebral vascular resistance (CVR) was calculated from the formula $CVR = \frac{MABP}{CBF}$ (7). Cerebral oxygen consumption (CMR) was determined by multiplying the CBF by the difference in oxygen content of the arterial and internal jugular blood.

Prior to operation a control cerebral blood flow measurement was made. At an interval of three to seven days following bilateral stellate ganglionectomy the studies were repeated. The patients were afebrile and received no premedication on either occasion. Earlier postoperative observations were precluded by the fact that general systemic changes (pulmonary effects and fever) would have obscured the results. This was unfortunate since it is well known that most vessels tend to regain a portion of their tone within several days after sympathectomy.

## RESULTS

The most striking finding in the series as a whole was the significant fall of CVR following bilateral stellate ganglionectomy, from a mean value of 2.6 to 2.0 resistance units ($p < .02$). There was not so clear an increase in the mean CBF from 45.6 to 55.6 cc./100 g./min. ($p > .05$) and in the CMR from 2.49 to 2.90 cc./100 g./min. ($p < .9$). Average values for the mean arterial blood pressure,

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[1] This investigation was supported in part by a research grant from the National Institutes of Health, Public Health Service.

pulse rate, cerebral respiratory quotient, jugular and arterial pH and $CO_2$ tensions were relatively unaffected by the operative procedure.

Both internal jugular and arterial oxygen content were significantly ($p < .02$) decreased (by 1.3 Vol. %) and the carbon dioxide content increased (3 Vol. %) following the bilateral removal of the stellate ganglia. These changes are probably due to a fall in the hematocrit postoperatively, and in one patient in whom the hematocrit was determined pre- and postoperatively such reduction did occur. The lowered hemoglobin content of the blood would result in reduction of the blood oxygen content. Blood with a lowered hematocrit would contain relatively greater amounts of plasma and since plasma does have a higher $CO_2$ content than red cells one would expect the $CO_2$ content of blood with a reduced hematocrit to be higher. However, since the $CO_2$ tension of the blood was unaltered by operation the increased $CO_2$ content of the blood could not be the critical factor in altering the cerebrovascular resistance postoperatively. Indeed, there is no correlation whatsoever between the changes in CVR with changes in any of the blood gas contents.

It is apparent that patients with initially slow CBF obtained the greatest reduction in CVR from the operation, and as a matter of fact an excellent correlation (Figure 1) exists between the preoperative CBF and amount of decrease in CVR ($r = .86$).

Almost as good correlation (Figure 2) exists between the preoperative CVR and the fall in CVR ($r = .75$).

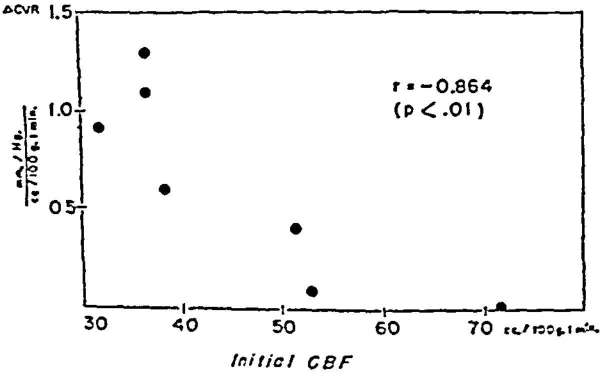

FIG. 1. RELATIONSHIP OF FALL IN CEREBROVASCULAR RESISTANCE FOLLOWING BILATERAL STELLECTOMY AND INITIAL CEREBRAL BLOOD FLOW

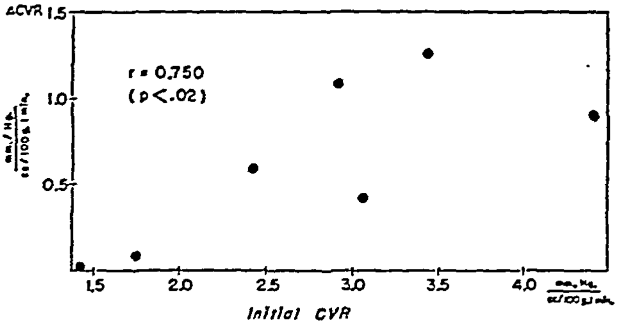

FIG. 2. RELATIONSHIP OF FALL IN CEREBROVASCULAR RESISTANCE FOLLOWING BILATERAL STELLECTOMY AND INITIAL CEREBROVASCULAR RESISTANCE

## DISCUSSION

Our data confirm previous findings, chiefly in animals, that under normal conditions the intrinsic tone of the cerebral vessels is not mediated via the autonomic nervous system fibers passing through the stellate ganglia. However, the clear effect of stellectomy in reducing the CVR in patients with abnormally slow CBF and increased CVR would indicate that, in pathologic states with increased CVR, at least a portion of this increased resistance can be due to spasm mediated over sympathetic reflexes.

The element of spasm maintained reflexly can evidently be considerably greater when induced by physiological means in the intact human than when produced in experimental animals by external, even though direct, stimulation of the sympathetic nerves.

The question whether the blood in the internal jugular bulb is significantly contaminated with drainage from the face and the scalp after stellate ganglionectomy and thereby influences our calculations of the cerebral blood flow and other cerebral circulatory functions is a pertinent one. Observations which demonstrated a very minor contamination of internal jugular bulb blood by extracerebral drainage were made on intact individuals (10). However, one would expect, if this error were introduced by the procedure of stellectomy, that the arteriovenous oxygen differences and respiratory quotients would be materially altered after operation. This in fact did not occur.

The discrepancy of our results with those reported by Harmel, Hafkenschiel, Austin, Crumpton and Kety (6) probably is due to the fact that

TABLE I

*Effect of bilateral stellate ganglionectomy on blood gas constituents*

| Patient | Age | | Blood $CO_2$ content (*Vol. %*) | | | | Blood $CO_2$ tension (*mm. Hg*) | | | | Blood $O_2$ content (*Vol. %*) | | | | Blood pH | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Arterial | | Int. jugular | | Arterial | | Int. jugular | | Arterial | | Int. jugular | | Arterial | | Int. jugular | |
| | | | I | II | I | II | I | II | I | II | I | II | I | II | I | II | I | II |
| L. S. | 38 | Post-enceph. Parkinsonism | 48.4 | 51.7 | 53.5 | 55.4 | 41 | 41 | 48 | 44 | 17.3 | 15.4 | 11.8 | 10.8 | 7.38 | 7.41 | 7.33 | 7.38 |
| M. M. | 51 | Post-enceph. Parkinsonism | 49.9 | 57.2 | 55.0 | 61.3 | 45 | 45 | 53 | 51 | 14.3 | 12.4 | 9.0 | 8.2 | 7.34 | 7.40 | 7.29 | 7.35 |
| J. W. | 27 | Post-enceph. Parkinsonism | 48.3 | 51.8 | 52.8 | 55.3 | 38 | 42 | 46 | 47 | 18.2 | 15.9 | 12.8 | 12.1 | 7.41 | 7.39 | 7.35 | 7.35 |
| W. N. | 42 | Post-enceph. Parkinsonism | 50.3 | 53.2 | 54.5 | 58.7 | — | — | — | — | 17.7 | 15.9 | 13.0 | 10.3 | — | — | — | — |
| A. A. | 43 | Essential hypertension | 51.0 | 53.8 | 55.9 | 59.6 | — | — | — | — | 14.0 | 13.1 | 8.8 | 7.0 | — | — | — | — |
| R. H. | 51 | Cerebral atrophy | 47.6 | 47.3 | 52.5 | 54.5 | 38 | 38 | 44 | 46 | 20.4 | 19.9 | 14.2 | 11.9 | 7.44 | 7.42 | 7.38 | 7.37 |
| B. D. | 59 | Cerebral arteriosclerosis | 50.2 | 51.1 | 55.6 | 56.5 | 44 | 44 | 52 | 50 | 16.5 | 15.3 | 11.1 | 9.6 | 7.37 | 7.38 | 7.31 | 7.34 |
| Mean | 44 | | 49.4 | 52.3* | 54.3 | 57.3* | 41 | 42 | 49 | 48 | 16.9 | 15.4* | 11.2 | 10.0* | 7.39 | 7.40 | 7.34 | 7.36 |

I—Preoperative study
II—Post-bilateral stellate ganglionectomy study
* Denotes significant difference ($p < .02$)

the latter observers did not study patients with an initially reduced cerebrovascular resistance and cerebral blood flow. Scheinberg (8) did study such patients but performed only unilateral stellate blocks. Our results are based upon bilateral removal of the stellate ganglion.

We did not have the opportunity to study patients with acute cerebrovascular accidents. However, the marked reflex cerebral vasoconstriction demonstrated in some of our patients makes the existence of such a mechanism plausible in some cases of apoplexy and would explain the beneficial effects reported from stellate ganglia block in such instances. It also provides a rationale for cervical sympathectomy at the time of carotid ligation to overcome vasospasm that might result from reflex mechanisms brought into play, in some patients, by the ligation.

TABLE II

*Effect of bilateral stellectomy on cerebral circulatory functions*

| Patient | (CBF) Cerebral blood flow (*cc./100 g./min.*) | | ($CMRO_2$) Cerebral $O_2$ consumption (*cc./100 g./min.*) | | (CVR) Cerebral vascular resistance $\frac{mm.\ Hg}{cc./100\ g./min.}$ | | (MABP) Mean arterial blood pressure (*mm. Hg*) | | (A–V $O_2$) Arterio-ven. oxygen difference (*Vol. %*) | | (R.Q.) Cerebral respiratory quotient | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | I | II | I | II | I | II | I | II | I | II | I | II |
| L. S. | 37 | 50 | 2.0 | 2.3 | 3.2 | 1.9 | 119 | 95 | 5.5 | 4.6 | .93 | .80 |
| M. M. | 37 | 69 | 2.0 | 2.9 | 2.7 | 1.6 | 100 | 109 | 5.3 | 4.2 | .96 | .98 |
| J. W. | 71 | 68 | 3.8 | 2.6 | 1.4 | 1.4 | 99 | 96 | 5.4 | 3.8 | .86 | .92 |
| W. N. | 39 | 54 | 1.8 | 3.0 | 2.2 | 1.6 | 87 | 88 | 4.7 | 5.6 | .90 | .98 |
| A. A. | 51 | 55 | 2.7 | 3.4 | 2.8 | 2.4 | 141 | 132 | 5.2 | 6.1 | .94 | .95 |
| R. H. | 31 | 38 | 1.9 | 3.0 | 4.2 | 3.3 | 131 | 126 | 6.2 | 8.0 | .79 | .90 |
| B. D. | 53 | 55 | 2.9 | 3.1 | 1.8 | 1.7 | 98 | 94 | 5.4 | 5.7 | 1.00 | .95 |
| Mean | 45.6 | 55.6 | 2.44 | 2.90 | 2.61 | 1.99* | 111 | 106 | 5.4 | 5.4 | .91 | .93 |

I—Preoperative study
II—Post-bilateral stellate ganglionectomy study
* Denotes significant difference ($p < .02$)

## SUMMARY

The cerebral circulation was studied in a series of seven patients before and after bilateral stellate ganglionectomy. A significant decrease in cerebrovascular resistance was found to result from stellate ganglionectomy. This decrease in cerebrovascular resistance following operation appears to be greatest in those patients with the initially slowest cerebral blood flow and initially most marked increases in cerebrovascular resistance.

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# THE EFFECT OF VENOUS AND ARTERIAL OCCLUSION, AND SYMPATHETIC NERVE TONE, ON DIGITAL BLOOD FLOW[1]

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(Submitted for publication July 13, 1950; accepted, October 30, 1950)

The normal range described originally (1) for blood flow in the fingertip, measured calorimetrically after vasodilatation by indirect heating, has been confirmed by Capriglione and Duque (2). Corrections for specific heat, gravity and temperature of blood (3), and the reinforcement of the effect of indirect heating by the intravenous administration of TEAC, subsequently raised the level of both normal and abnormal readings slightly. The normal range for the fingertip now is 0.25 to 0.38 cc./sq.cm. of skin/min., and for the toe 0.15 to 0.29 cc./sq.cm./min. (4). Venous obstruction plethysmography of the fingertip gives a normal range after indirect heating which is somewhat wider [6 to 12 cc./10 cc. of finger volume/min. (5)]. What is more, we have often observed considerable differences between single digital plethysmographic and digital calorimetric readings in the same subject under presumably similar conditions. Such discrepancies have also been noted between calorimetric and plethysmographic studies of the hand (6) especially at intermediate rates of flow, although the differences are ascribed to the "smoothing out" by the calorimeter of rapid fluctuations in flow recorded plethysmographically.

Hoobler, Avera, Little, Peet, and Bassett (7), employing the foot plethysmograph, have described considerably greater increases in blood flow during caudal anesthesia or after sympathectomy than after indirect heating, or after the intravenous injection of TEAC. These differences were attributed to the failure of indirect heat or TEAC to release sympathetic nerve tone completely. Arnott and Macfie (8), however, measuring blood flow calorimetrically, found that blocking the ulnar nerve with procaine did not increase flow in the fifth finger if sympathetic nerve tone had been previously released by indirect heat.

In an attempt to reconcile some of these discrepancies, the calorimetric method was subjected to exhaustive critical evaluation, the results of which are reported elsewhere (3). The assumptions involved in the method were sustained. It therefore seemed necessary to investigate some of the assumptions inherent in the plethysmographic method (9), the most vulnerable of these being the assumption that venous obstruction traps all the blood in the part. It also became necessary to investigate the completeness of release of sympathetic nerve tone by indirect heating supplemented by TEAC in the skin of the great toe.

## METHODS

Accordingly, a series of experiments were set up wherein blood flow was measured calorimetrically after arterial and after venous obstruction, using heat supplemented by TEAC to release sympathetic nerve tone. Patients without vascular disease, and some with mild residua of "trench foot," were used as subjects. Heat was administered to the trunk by a cradle baker for from 30 to 45 minutes until positive heat balance was attained as manifested by generalized sweating. Then, TEAC (5 mg./Kg) was injected intravenously. Flow studies were begun approximately 10 minutes after the injection. For the great toe the compressing cuff was at the ankle, whereas for the fingertip (third or fourth) a Gaertner capsule was used at the proximal phalangeal joint. The arterial obstruction pressure varied between 180 and 220 and the venous obstruction pressure between 40 and 50 mm. Hg. Only those results were tabulated in which the flow within a minute after obstruction was at or near the original level. In approximately 10 per cent of subjects, venous obstruction produced a decrease in flow, which persisted long after release of pressure, indicating reflex vasoconstriction (see Figure 1). That psychic factors can "break through" indirect heat and less commonly through indirect heat supplemented by TEAC to produce reflex vasoconstriction is well known (10) and is demonstrated in Figure 2.

In addition, blood flow in the great toe measured calorimetrically after indirect heating and TEAC was com-

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[1] Reviewed in the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions published by the authors are the result of their own study and do not necessarily reflect the opinion or the policy of the Veterans Administration.

This study was aided by Grants from the American Heart Association and the Research Committee of the American Medical Association.

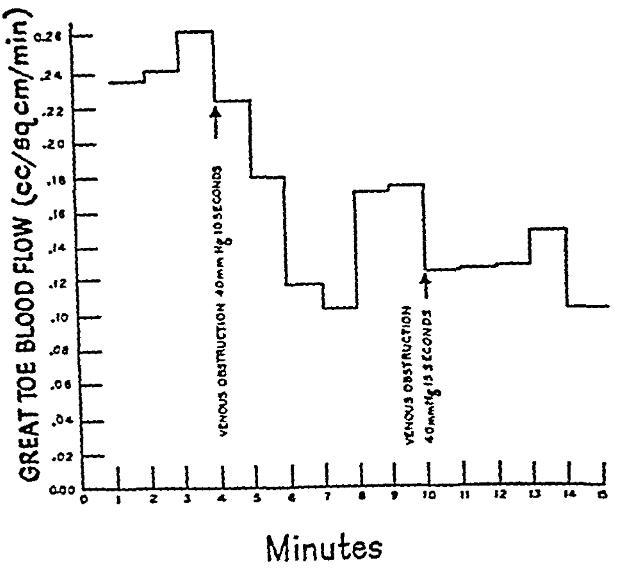

FIG. 1. REFLEX VASOCONSTRICTION PRODUCED BY VENOUS OCCLUSION

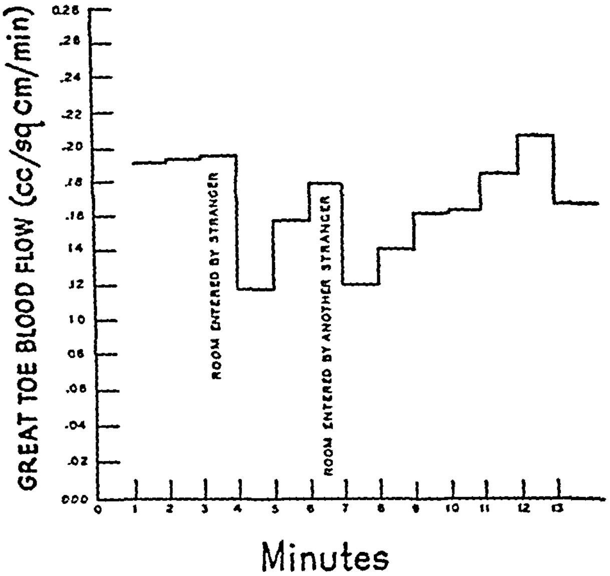

FIG. 2. THE EFFECT OF PSYCHIC STIMULI ON DIGITAL BLOOD FLOW (TOE)

pared with blood flow measured during spinal anesthesia in 12 patients. Spinal anesthesia was administered to these patients for surgical operations such as hernioplasty or hemorrhoidectomy. Anesthesia was considered complete when the patient became insensitive to pin prick and was unable to move his lower extremities. One additional patient suffering from causalgia, was studied pre-and two days post-sympathectomy.

## RESULTS

The changes in blood flow after arterial and venous obstruction, in typical experiments, are presented graphically in Figures 3A and B. The results in many experiments on upper as well as lower extremities (Tables I and II) confirmed these findings. It will be seen that venous obstruction for several minutes decreased blood flow *only slightly, and sometimes not at all*; whereas, arterial obstruction reduced blood flow in the fingertip or toe sharply, although not quite to the zero level. The heat of metabolism was calculated

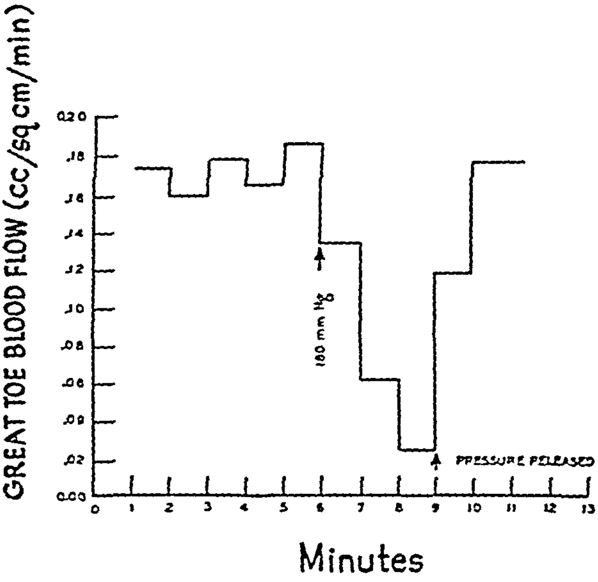

FIG. 3A. THE EFFECT OF ARTERIAL OCCLUSION ON DIGITAL BLOOD FLOW (TOE)

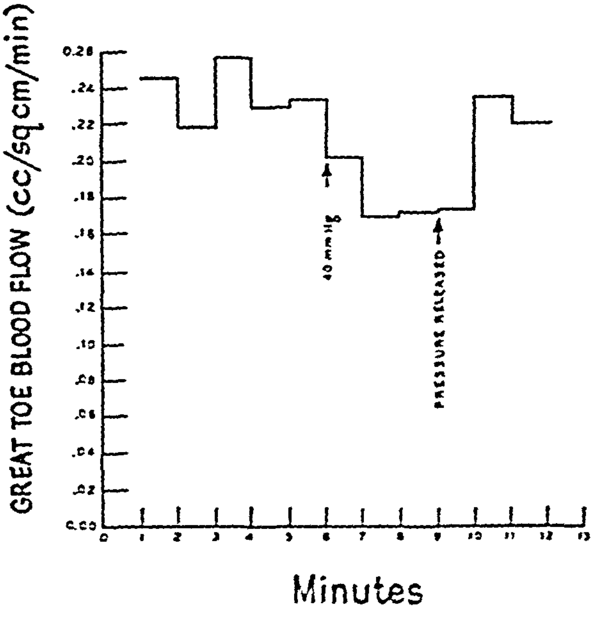

FIG. 3B. THE EFFECT OF VENOUS OCCLUSION ON DIGITAL BLOOD FLOW (TOE)

TABLE I

*The effect of arterial obstruction on digital blood flow*

| Digit | Time of obstruction | Flow before obstruction | Lowest flow during obstruction | Flow after obstruction | Maximum per cent change in flow |
|---|---|---|---|---|---|
| | *min.* | *cc./sq.cm./min.* | *cc./sq.cm./min.* | *cc./sq.cm./min.* | |
| *Great Toe* | 9 | 0.15 | 0.01 | 0.16 | −93 |
| | 6 | 0.18 | 0.02 | 0.15 | −89 |
| | 6 | 0.16 | 0.02 | 0.14 | −88 |
| | 3 | 0.17 | 0.03 | 0.16 | −82 |
| | 3 | 0.18 | 0.07 | 0.21 | −61 |
| | 3 | 0.17 | 0.02 | 0.17 | −88 |
| | 3 | 0.26 | 0.09 | 0.25 | −65 |
| | 3 | 0.16 | 0.06 | 0.20 | −63 |
| | 3 | 0.18 | 0.04 | 0.20 | −78 |
| | 3 | 0.15 | 0.04 | 0.15 | −73 |
| | 3 | 0.17 | 0.07 | 0.12 | −59 |
| | 2 | 0.18 | 0.07 | 0.23 | −61 |
| | 2 | 0.19 | 0.06 | 0.17 | −68 |
| | ½ | 0.26 | 0.21 | 0.23 | −19 |
| | ½ | 0.23 | 0.18 | 0.23 | −22 |
| | ½ | 0.27 | 0.17 | 0.25 | −37 |
| | ½ | 0.20 | 0.14 | 0.20 | −30 |
| | ½ | 0.27 | 0.20 | 0.26 | −26 |
| | ½ | 0.17 | 0.12 | 0.17 | −29 |
| | ½ | 0.24 | 0.17 | 0.27 | −29 |
| | ½ | 0.14 | 0.11 | 0.15 | −21 |
| *Finger* | 8 | 0.34 | 0.07 | 0.28 | −79 |
| | 3 | 0.32 | 0.03 | 0.32 | −91 |
| | 3 | 0.24 | 0.04 | 0.25 | −83 |
| | 3 | 0.32 | 0.03 | 0.28 | −91 |
| | 3 | 0.12 | 0.03 | 0.11 | −75 |
| | 3 | 0.26 | 0.04 | 0.24 | −85 |
| | 3 | 0.39 | 0.05 | 0.38 | −87 |
| | ½ | 0.31 | 0.23 | 0.34 | −26 |
| | ½ | 0.21 | 0.16 | 0.23 | −29 |
| | ½ | 0.29 | 0.23 | 0.29 | −21 |
| | ½ | 0.27 | 0.21 | 0.30 | −22 |
| | ½ | 0.24 | 0.20 | 0.24 | −17 |
| | ½ | 0.32 | 0.22 | 0.33 | −31 |
| | ½ | 0.30 | 0.22 | 0.33 | −27 |

to be too small to be measured calorimetrically (3) and hence was not considered responsible for the residual heat elimination during arterial obstruction. With digital vasodilatation, some blood reached the fingertip or toe, probably via deep bone channels, even when the arteries were obstructed at several levels. To obliterate blood flow through the fingertip or toe completely, it was necessary to supplement arterial obstruction at the level of the ankle and toe, or at the level of upper arm, wrist and finger, by reflex vasoconstriction for several minutes (3). Several experiments, in which venous and arterial obstruction were employed for only 30 seconds or less, also revealed less decrease in flow during venous than during arterial obstruction, although the differences were less striking. The "lag" factor of the digital calorimeter, however, is approximately one minute and each of the series of flow determinations made in any patient covers a period of one minute. It would therefore be expected that such differences as occur with obstruction over a fraction of a minute would be "damped" to some extent. The lower venous pressure in the initial phase of venous obstruction might also be a "damping" factor.

In Table III, blood flow in the great toe measured calorimetrically after indirect heating supplemented by TEAC is compared with blood flow

TABLE II

*The effect of venous obstruction on digital blood flow*

| Digit | Time of obstruction | Flow before obstruction | Lowest flow during obstruction | Flow after obstruction | Maximum per cent change in flow |
|---|---|---|---|---|---|
| | *min.* | *cc./sq.cm./min.* | *cc./sq.cm./min.* | *cc./sq.cm./min.* | |
| *Great Toe* | 9 | 0.23 | 0.14 | 0.24 | −39 |
| | 4 | 0.21 | 0.15 | 0.30 | −29 |
| | 4 | 0.08 | 0.05 | 0.08 | −38 |
| | 4 | 0.17 | 0.14 | 0.18 | −18 |
| | 3 | 0.15 | 0.12 | 0.14 | −20 |
| | 3 | 0.05 | 0.04 | 0.09 | −20 |
| | 3 | 0.17 | 0.11 | 0.15 | −35 |
| | 3 | 0.23 | 0.17 | 0.23 | −26 |
| | 3 | 0.15 | 0.16 | 0.16 | + 7 |
| | 3 | 0.25 | 0.18 | 0.28 | −28 |
| | 2 | 0.23 | 0.23 | 0.26 | 0 |
| | 2 | 0.13 | 0.08 | 0.10 | −38 |
| | 2 | 0.21 | 0.18 | 0.24 | −14 |
| | 2 | 0.16 | 0.13 | 0.16 | −19 |
| | 2 | 0.16 | 0.13 | 0.15 | −19 |
| | ½ | 0.06 | 0.06 | 0.06 | 0 |
| | ½ | 0.15 | 0.12 | 0.16 | −20 |
| | ½ | 0.12 | 0.13 | 0.13 | + 8 |
| | ½ | 0.19 | 0.18 | 0.18 | − 5 |
| | ½ | 0.28 | 0.12 | 0.30 | −57 |
| | ½ | 0.19 | 0.15 | 0.19 | −21 |
| | ½ | 0.14 | 0.14 | 0.13 | 0 |
| | ½ | 0.11 | 0.11 | 0.11 | 0 |
| | ½ | 0.17 | 0.17 | 0.17 | 0 |
| | ½ | 0.16 | 0.16 | 0.18 | 0 |
| *Finger* | 3 | 0.28 | 0.26 | 0.32 | −32 |
| | 3 | 0.41 | 0.22 | 0.51 | −46 |
| | 3 | 0.16 | 0.06 | 0.12 | −63 |
| | 3 | 0.20 | 0.18 | 0.22 | −10 |
| | 3 | 0.38 | 0.24 | 0.39 | −37 |
| | 3 | 0.15 | 0.08 | 0.14 | −47 |
| | 3 | 0.23 | 0.18 | 0.19 | −22 |
| | ½ | 0.30 | 0.26 | 0.28 | −13 |
| | ½ | 0.24 | 0.21 | 0.24 | −13 |
| | ½ | 0.35 | 0.31 | 0.38 | −11 |
| | ½ | 0.42 | 0.36 | 0.41 | −14 |
| | ½ | 0.16 | 0.15 | 0.16 | −06 |
| | ½ | 0.26 | 0.23 | 0.26 | −12 |
| | ½ | 0.28 | 0.24 | 0.28 | −14 |
| | ½ | 0.39 | 0.36 | 0.40 | −08 |
| | ½ | 0.25 | 0.21 | 0.23 | −16 |
| | ⅓ | 0.25 | 0.22 | 0.25 | −12 |
| | ⅓ | 0.29 | 0.25 | 0.29 | −14 |
| | ¼ | 0.28 | 0.22 | 0.27 | −21 |

TABLE III

| Patient | Age and sex | Indirect heating and TEAC | | Spinal anesthesia or sympathectomy | | |
|---|---|---|---|---|---|---|
| | | Brachial blood pressure | Blood flow | Dosage pontocaine | Brachial blood pressure | Blood flow |
| | | mm. Hg | cc./sq.cm./min. | | mm. Hg | cc./sq.cm./min. |
| 1 | 28 M | 130/80 | 0.15 | 13 mg. | 143/85 | 0.15 |
| 2 | 60 M | 128/100 | 0.19 | 20 mg. | 100/80 | 0.12 |
| 3 | 15 M | 108/64 | 0.25 | 8 mg. | 118/60 | 0.08 |
| 4 | 59 F | 204/84 | 0.14 | 12 mg. | 160/80 | 0.14 |
| 5 | 66 M | 132/60 | 0.15 | 15 mg. | 95/75 | 0.10 |
| 6 | 48 F | 108/64 | 0.18 | 16 mg. | 100/70 | 0.14 |
| 7 | 55 F | 96/70 | 0.16 | 15 mg. | 95/75 | 0.19 |
| 8 | 42 M | 92/76 | 0.21 | 16 mg. | 110/70 | 0.10 |
| 9 | 24 M | 88/70 | 0.14 | 15 mg. | 90/60 | 0.15 |
| 10 | 44 F | 112/72 | 0.23 | 15 mg. | 85/70 | 0.17 |
| 11 | 29 M | 82/52 | 0.15 | 16 mg. | 80/50 | 0.07 |
| 12 | 29 M | 116/80 | 0.15 | 12 mg. | 108/80 | 0.20 |
| 13 | 22 M | 130/90 | 0.21 | 2 days after sympathectomy | 110/72 | 0.19 |

during spinal anesthesia in 12 cases and after sympathectomy in one case. With the calorimetric technique blood flow during spinal anesthesia or after sympathectomy was no greater, and in many cases considerably less, than after indirect heating reinforced by TEAC. A decreased systemic blood pressure or circulating vasoconstrictive substances, such as adrenalin, released by the apprehension prior to an operation, might account for the depression of blood flow during spinal anesthesia in those cases in which it occurred. Page, Prince, and Reinhard (11) have shown that circulating vasoconstrictive substances are released from the adrenal medullae and the liver of animals by the intravenous administration of large doses of TEAC. Since the normal range of variation in blood flow is shifted only slightly by supplementing indirect heating by the administration of TEAC intravenously (4) it is unlikely that significant humoral vasoconstriction was induced in our patients by TEAC, in the dosage used.

## DISCUSSION

It is apparent from these experiments that prolonged venous obstruction is incomplete because of considerable reflux of blood probably via deep veins. This reflux may occasionally be great enough to accommodate the total influx from the arteries. Even with 30 seconds of venous obstruction, all the blood is not trapped in the finger or toe. We have no evidence bearing on the first five or 10 seconds of venous obstruction where digital plethysmographic slopes are usually drawn. The calorimetric lag is too great to make successful comparisons of flow at such short intervals. It must be pointed out, however, that reflux of blood may occur even during this initial phase of obstruction, and may produce a variable error in the measurement of blood flow. It has also been demonstrated (12) that at high rates of flow increased pulse volume makes it more difficult to draw accurate digital plethysmographic slopes. In contrast, it is at such high rates of flow that the digital calorimeter becomes most reliable (3). In addition to these factors, it is clear that reflexes induced psychically, or by the very act of venous obstruction, may "break through" the release of sympathetic tone by heating alone and less commonly by heating together with intravenously administered TEAC to distort flow measurements dependent on such obstruction. Since it is less likely that such reflexes can "break through" spinal anesthesia, this factor would also confuse comparison between procedures for releasing sympathetic nerve tone.

In the studies with the foot plethysmograph (7) caudal anesthesia or sympathectomy was compared with heat or TEAC rather than a combination of the two. The combination may be more effective than either of these agents alone in releasing sympathetic nerve tone in the lower extremities. What is more, there may be differences between the reactions of deep blood vessels and those of the skin (7) making comparison between the results of foot plethysmography and digital calorimetry difficult. The fact, however, that spinal anesthesia is no more effective than indirect heating supplemented by TEAC in releasing sympathetic nerve tone, if the calorimetric technique is used, indicates that the latter procedure releases such tone effectively at least in the skin of the toe.

## SUMMARY AND CONCLUSIONS

Spinal anesthesia produces no greater release of sympathetic nerve tone in the great toe than indirect heating supplemented by TEAC when blood flow in the skin is measured calorimetrically. Factors responsible for discrepancies between plethysmographic and calorimetric data were investigated and are discussed.

ACKNOWLEDGMENT

Advice of Dr. S. Feitelberg and technical assistance of Miss S. Lichtenberg, Mr. R. E. Schwartz and Mr. G. Grossinger are gratefully acknowledged.

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# CHEMICAL, CLINICAL, AND IMMUNOLOGICAL STUDIES ON THE PRODUCTS OF HUMAN PLASMA FRACTIONATION. XL. QUANTITATIVE SEPARATION AND DETERMINATION OF THE PROTEIN COMPONENTS IN SMALL AMOUNTS OF NORMAL HUMAN PLASMA[1]

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(Submitted for publication September 25, 1950; accepted, October 30, 1950)

The need for improved methods for the determination and characterization of the protein components of human blood plasma requires no emphasis. The commonly employed methods for the separation of plasma proteins on the basis of solubility give little detailed information and generally yield poor separations (1). Likewise, the method of electrophoretic analysis, although of unquestioned value, will fail to differentiate between components of widely differing chemical properties and physiological functions if they happen to have the same mobility in an electric field.

The present communication describes the adaptation of Method 10 of plasma fractionation developed by Cohn and his associates (2) for the quantitative estimation of certain of the protein and lipoprotein components in small quantities of normal human plasma. The fractionation is carried out at low temperatures using low concentrations of ethanol and of various salts, resulting in the concentration of each protein component in an undenatured state almost exclusively in one fraction or another.

In the present system of analysis, samples of 5 ml. of plasma have been separated by filtration into four primary fractions (Figure 1). The concentrations of the serum albumins, $\gamma$-globulins, $\alpha$-lipoproteins, and $\beta$-lipoproteins were measured, together amounting to about 75% of the total proteins of normal human plasma. Data have also been obtained on the distribution of protein-bound carbohydrate (hexose) among the fractions, but the information is not yet available which would make it possible to calculate the quantities of specific glycoproteins and mucoproteins.

Although the present communication deals with the determination of only four major components of normal plasma, the system of separation has been designed to allow the incorporation of procedures for the isolation and measurement of other components as soon as methods have been worked out. In addition to quantitative analysis of the major protein components in the plasma the proposed method will facilitate the quantitative estimation of trace components, because each fraction contains certain components in higher concentration and in simpler mixtures than whole plasma. Because the proteins are present in various fractions in an unaltered state their biological activities remain unimpaired and can be measured. The method can, furthermore, be used on abnormal plasmas for the quantitative determination of various proteins present in abnormal quantities in the plasma of patients with various diseases. Such quantitative analyses of abnormal plasmas are being carried out at present and will be published in a future communication. Recognition and characterization of pathological proteins, as they are present in several diseases, such as multiple myeloma and lupus erythematosus, will be facilitated by this method.

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[1] This work was supported by grants from the National Institutes of Health, from industry, funds at the disposal of Harvard University, and by aid from the Harry Helfman Pemphigus Research Fund, the Milton Fund, and other sources. This paper is No. 91 in the series "Studies on the Plasma Proteins" from blood collected by the American Red Cross, on products developed by the University Laboratory of Physical Chemistry Related to Medicine and Public Health, Harvard University.

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The method has been designed to be as simple and rapid as possible without sacrificing accuracy. All operations, including the separations of precipitates by filtration under vacuum, are carried out in the same cold bath. The separations of six samples of plasma into the four primary fractions can normally be completed in one day by a single worker. The buffer solutions employed were chosen so that for each step stock reagents are added in standard amounts without the necessity of performing tedious pH adjustments. Accurate separations have been made possible by the use of liberal quantities of buffer solutions to wash the precipitates on the filters. The use of the buffer solutions for washing has the added advantage that small errors in the pH adjustment at any one step are corrected before subsequent separations are undertaken, and in this way such errors are prevented from accumulating.

### SYSTEM OF PLASMA ANALYSIS

Method 10 of plasma analysis employs a system in which five or more variables are critically controlled: pH, ethanol concentration, ionic strength, protein concentration, temperature and, in some instances, concentration of dipolar ions or of divalent metallic or organic ions.[5] The conditions at which these variables are fixed, and the components known to be concentrated in the different fractions are given in Figure 1.

In the *first step* of the procedure, many of the largest and least soluble of the plasma proteins are rendered completely insoluble, and are separated in Fraction I + II + III. The extremely low solubility of the proteins of Fraction I + II + III under the conditions of this separation is due in part to the formation of insoluble salt-like complexes between proteins of opposite net charge (2). The $\gamma$-globulins, most notably, owe their insolubility to combination with $\beta$-lipoproteins and other $\alpha$-globulins and $\beta$-globulins, including caeruloplasmin. In a sense, the components naturally present in the plasma are used as reagents to effect their own quantitative separation. It will be shown below that wide variations in the concentrations of $\beta$-lipoproteins and $\gamma$-globulins do not interfere with the completeness of the separation of Fraction I + II + III from Fraction IV + V + VI.

The *second step* of the procedure involves the precipitation in Fraction IV + V of nearly all the proteins which remained in solution after the initial precipitation of Fraction I + II + III. In this way almost all the plasma proteins are reduced to the more stable solid state within a short time after the beginning of the fractionation. The precipitation is effected by the addition of a small amount of zinc acetate without changing the other variables. The proteins precipitated as insoluble zinc salts include the serum albumins, $\beta_1$-metal combining protein, $\alpha_2$-glycoproteins, $\alpha_2$-mucoproteins, $\alpha$-lipoproteins, iodoproteins, choline esterase and alkaline phosphatase. The zinc salts are readily soluble in distilled water and in a variety of buffer solutions. Analytical determinations for serum albumins and $\alpha$-lipoproteins were usually made on redissolved Fraction IV + V, although for certain purposes measurements could be made directly on the solution of Fraction IV + V + VI. However, for the determination of protein-bound carbohydrate (as hexose) it is necessary to separate Fraction IV + V and then to precipitate Fraction VI, leaving the free carbohydrate (blood sugar) in solution. Serum albumins are estimated as methemalbumin (5), and the $\alpha$-lipoproteins from determinations of cholesterol and lipid phosphorus.

Fraction VI, which remains in solution after the precipitation of Fraction IV + V, contains traces of the proteins of Fraction IV + V, and in addition an $\alpha_1$-acid glycoprotein, small amounts of $\alpha_2$-globulins and $\beta_1$-globulins, as well as other small proteins and peptides, urea, and glucose. The protein and certain peptide constituents of this fraction are precipitated by the addition of barium acetate and an increase in the concentration of ethanol.

The *third step* of the procedure serves to extract from Fraction I + II + III almost all the $\gamma$-globulins in a nearly pure form, as judged by electrophoretic analysis of Fraction II. To accomplish this separation, it was found necessary to add the dipolar ion glycine to decompose the complexes in which the $\gamma$-globulins are held. The ethanol concentration of 15% and the low ionic strength used

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[5] For more detailed information about the role of these variables in protein separations, see Cohn and his colleagues (2, 3) and the review by Edsall (4).

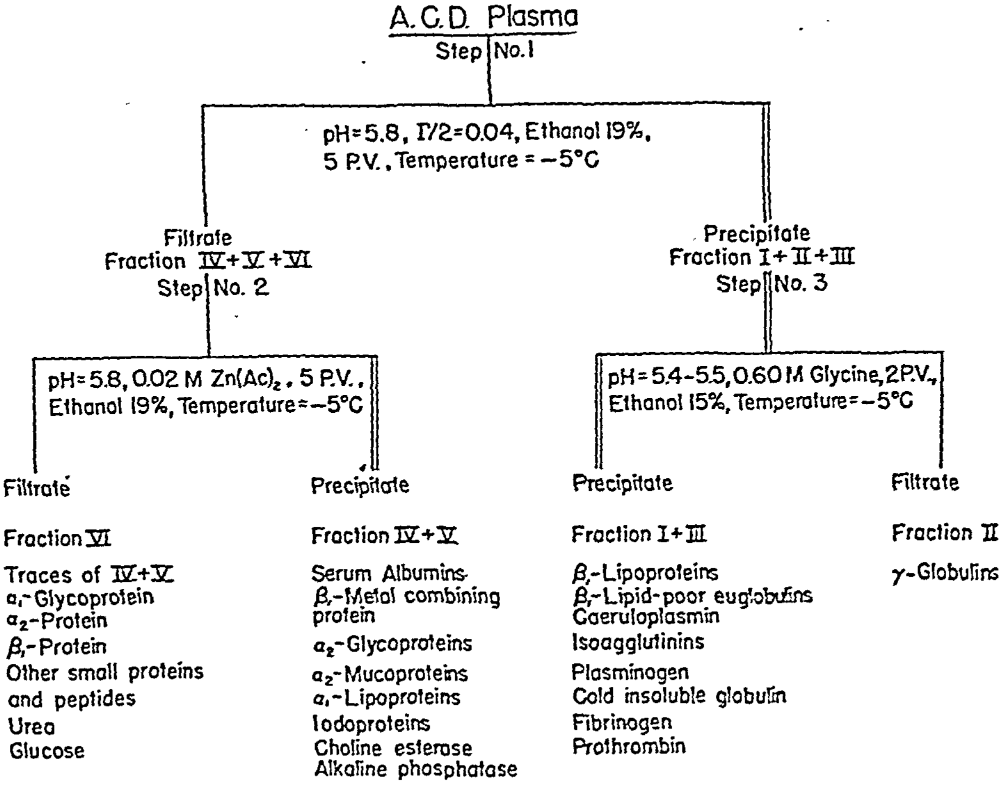

FIG. 1. PROTEIN COMPONENTS CONCENTRATED IN THE FOUR PRIMARY FRACTIONS

during the extraction prevents solution of more than traces of β-globulins.

The yield of protein in Fraction II is obtained by direct measurement with the biuret reaction on an aliquot of the solution. When concentration of the extract is required prior to electrophoretic, ultracentrifugal, or other analyses, the γ-globulins are quantitatively reprecipitated by the addition either of sodium tartrate or of a mixture of sodium sulfate and zinc acetate, accompanied by an increase in concentration of ethanol. The use of the divalent anions makes it unnecessary to increase the ethanol concentration to the point where glycine would precipitate along with the γ-globulins. The precipitates obtained in this way are readily soluble in distilled water or a wide variety of salt solutions.

Fraction I + III, which remains insoluble after the extraction of the γ-globulins, contains β-lipoproteins, caeruloplasmin, isoagglutinins, most of the components concerned with blood clotting, and a number of α-globulins and β-globulins whose characteristics are not yet well understood. The use of the technique of fractional extraction for the removal of the γ-globulins serves to keep the components of Fraction I + III insoluble from the time that they are precipitated in the initial step of the procedure until they are redissolved for analysis at the very end of the operation. In the solid state these components are quite stable and are protected from various changes, chemical and enzymatic, which occur rapidly in solution. The yield of β-lipoproteins in Fraction I + III is estimated from cholesterol and lipid phosphorus determinations on the redissolved fraction.

## MATERIALS AND METHODS

### *Collection of Blood*

Blood was obtained from male subjects by venipuncture with a syringe containing 1.5 ml. of acid citrate dextrose solution (A.C.D.) for each 8.5 ml. of blood to be drawn. The blood cells were removed by centrifugation to obtain the plasma, designated as "A.C.D. plasma," which was used for the separations and analyses.[e] The acid citrate dextrose solution contained per liter 26.7 g. trisodium citrate ($5\frac{1}{2}$ $H_2O$), 8.0 g. citric acid monohydrate, and 22 g. dextrose. At the same time, for the purpose of a reference determination of total protein, approximately 5 ml. of blood were drawn with a dry syringe, and placed in a tube containing a small amount of heparin powder.

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[e] The tendency of the clot to entrap certain proteins to a larger extent than others prompted our decision not to apply the separation method to serum (6).

### *Apparatus*

The fractionation was carried out in a −5°C. bath containing 50% by volume of ethanol, provided with an effective stirrer, and deep enough to submerge the filtration apparatus to the required depth.[7] For the filtration a sintered glass filter cup with a maximum porosity of 14 microns and a capacity of 60 ml. was used.[8, 9] This filter cup was connected to a 100 ml. round-bottom collecting tube by a two-hole rubber stopper. The glass stem of the filter cup entered the collecting tube through one hole of the stopper. A suction system was connected through the other hole of the stopper. The filter cups and attached collecting tubes were held in the bath by a rigid amount of sufficient size to accommodate 12 such filtration units. The availability of 12 filtration units allowed the processing of six specimens of blood plasma in one day.

A water aspirator was used to obtain vacuum. A regulating column of mercury was employed to control the vacuum,[10] and a large carboy was placed in the suction line to minimize pressure variations due to the aspirator. A steady pressure of 38 cm. of mercury was found most effective.

Adequate cleaning of the glass filter cups as soon as possible after use is important. They were first washed with soap and water. Thereafter, saturated borax solution, dilute hydrochloric acid, distilled water, and 95% alcohol were drawn successively through the filters under negative pressure provided by a water aspirator. (If the filters were still plugged, several lots of a 2% aqueous solution of duponol were drawn through the filters prior to the use of the saturated borax solution.) It was found inadvisable to use any one glass filter cup for more than ten runs.

### *Reagents*

All reagents were freshly prepared from stock solutions at room temperature and precooled to −5° (or the freezing point of the mixture) before use. The stock solutions were made with C.P. grade reagents without further purification.

*Reagent A* contained 250 ml. 95% ethanol, and 2.4 ml. of sodium acetate buffer, and water to make 1 L. The stock sodium acetate buffer consisted of 200 ml. of 4 M sodium acetate and 400 ml. of 10 M acetic acid per L. This buffer diluted with distilled water 80 times had a pH of 4.00 ± 0.02 in a glass electrode at 25° ([3] footnote 68).

*Reagent A'* contained 200 ml. of 95% ethanol, 40 ml. of 1 M sodium acetate, 3.5 ml. of 1 M acetic acid, and water to make 1 L. The pH was 5.80 ± 0.05.

*Zinc Reagent* contained 54.8 g. of zinc acetate dihydrate which was first dissolved in about 500 ml. of water; to this solution 200 ml. of 95% ethanol were added, and then water to make 1 L. The solution should be kept at refrigerator temperature since at room temperature undesirable deposition of insoluble material occurs. The pH of this solution was 6.5 ± 0.1.

*Barium Reagent* contained 10.22 g. barium acetate, 640 ml. 95% ethanol, and water to make 1 L.

*Reagent B* contained 150 ml. 95% ethanol, 45 g. glycine, 3.8 ml. 1 M sodium acetate, 2.6 ml. 1 M acetic acid, and water to make 1 L. The pH was 5.20 ± 0.02.

*Reagent B'* contained 150 ml. of 95% ethanol, 45 g. glycine, 5 ml. of 1 M sodium acetate, 1.8 ml. 1 M acetic acid, and water to make 1 L. The pH was 5.40 − 5.45.

*Citrate Reagent* contained 9.0 g. sodium chloride, 7.1 g. trisodium citrate (5½ $H_2O$), and water to make 1 L.

*Tartrate Reagent* contained 13.8 g. disodium tartrate, 475 ml. 95% ethanol, and water to make 1 L.

*Sulfate Reagent* contained 8.5 g. anhydrous sodium sulfate, 4.4 g. zinc acetate dihydrate, 362 ml. of 95% ethanol, and water to make 1 L.

### *Separation Procedures*

*Step No. 1.* The initial precipitation of Fraction I + II + III, leaving Fraction IV + V + VI in solution, was performed as follows. Two ml. of precooled Reagent A were placed in the sintered glass filter cup which was immersed in the cold bath within 1 cm. of the lip. Five ml. of A.C.D. plasma precooled to 0–2° were added dropwise with stirring, followed by 18 ml. of Reagent A again added dropwise with good stirring.[11] After the addition was completed, the mixture was stirred for five minutes, and allowed to stand for 10 to 15 minutes before the vacuum was applied. The filtration required about 40 minutes.

The precipitate was then washed[12] by resuspending it in 25 ml. of Reagent A′. The precipitate was at first stirred to a thick uniform paste with a small amount of the reagent before the rest of the reagent was added with constant stirring. The collecting tube containing Fraction IV + V + VI was left in place to receive the washings. The vacuum was applied without delay. The filtration required about 30 minutes.

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[7] A 50 gallon capacity cold bath supplied by the American Instrument Company, Silver Spring, Md. was used. The internal dimensions are 21.75 × 37.75 × 15 inches, and the temperature was adjustable to within 0.5°C. over a temperature range between room temperature and −29°.

[8] These filter cups are made by the Corning Glass Works, and were obtained from Macalaster-Bicknell Co., Cambridge, Mass. Filter cups with a maximum porosity of 14 microns are marked M; those with a maximum porosity of 5 microns are marked F.

[9] Very rarely, it was found that the precipitate formed by the initial precipitation would pass through the filter. In such instances a sintered glass filter cup with a maximum porosity of 5 microns was used to obtain a clear filtrate.

[10] In recent work, satisfactory vacuum regulation has been obtained with a Cartesian Manostat No. 6, supplied by Emil Greiner Co., 22 N. Moore St., New York, N. Y.

[11] If the plasma was placed in the filter cup before any reagent was added, it was found that some of the plasma penetrated the pores of the filter before precipitation occurred. This resulted in considerable slowing of the filtration due to freezing of some of the plasma in the filter pores, and cloudiness of the filtrate due to the escape of some precipitable proteins through the filter. On the other hand, if larger volumes of reagent were added first, the plasma would be exposed initially to excessively acid conditions.

[12] Without washing, appreciable quantities of the soluble proteins belonging to Fraction IV + V + VI remained

*Step No. 2.* As soon as the filtration of the washing had been completed, the filtration apparatus was momentarily removed from the cold bath, the receiving flask transferred to a wire basket suspended in the cold bath, and a fresh receiving flask attached in its place. The filtration apparatus was immediately reimmersed. To the solution of Fraction IV + V + VI in the first receiving flask were added 4 ml. of the Zinc Reagent, causing copious precipitation. The resulting suspension was allowed to stand for 15 to 30 minutes in the cold bath (overnight was found permissible), and was then poured into a new filter cup which differed from the first only in that the maximum porosity was 5 microns instead of 14 microns. The last traces of the suspension in the first receiving flask were washed into the filter cup with the aid of a few ml. of 0.02 M zinc acetate dissolved in 19% ethanol. Filtration was carried out as before. The precipitate remaining on the filter constituted Fraction IV + V, and was redissolved either in 0.15 M sodium chloride or 0.02 M sodium citrate to a volume of 5 ml. for the purpose of analysis.

The filtrate, Fraction VI, contained so little protein that frequently it was not subjected to further isolation procedures. When desired, the proteins of Fraction VI were precipitated by the addition of an equal volume of Barium Reagent, and separated as before by filtration through a glass filter with a maximum porosity of 14 microns. The precipitate was washed with two 10 ml. portions of the following reagent: 5.47 g. barium acetate monohydrate, 4.39 g. zinc acetate dihydrate, 421 ml. 95% ethanol, and water to make 1 L. The precipitate was then dissolved in a mixture of 0.5 ml. Citrate Reagent and 4.5 ml. distilled water, and the resulting solution drawn through the filter. The filter was washed through with 2 ml. of 0.1 M sodium sulfate solution and the washings added directly to the dissolved Fraction VI. The Fraction VI solution was made up to 10 ml. with distilled water, and placed in a refrigerator for 30 minutes to allow the precipitate of barium sulfate to form. The precipitate was removed by a short centrifugation in an ordinary laboratory centrifuge before aliquots of the clear solution were taken for biuret and hexose analyses. If only the biuret determination was required, it was sufficient to dissolve the precipitate of Fraction VI in the diluted Citrate Reagent.

*Step No. 3.* The third step of the separation procedure involved the extraction of Fraction II from Fraction I + II + III, leaving Fraction I + III as residue. This separation was carried out concurrently with Step No. 2. Fraction I + II + III in the original filter cup was resuspended in 25 ml. of Reagent B. As in the previous washing with Reagent A′, only about 1 ml. of Reagent B was added at first, and a thick, uniform paste was prepared. Thereafter, the rest of the reagent was added slowly with constant stirring. The suspension was allowed to stand for one hour with occasional stirring before the vacuum was applied. The filtration required approximately two hours.

The residue was then washed by resuspending it in 10 ml. of Reagent B′. Filtration was started without delay. The washings were filtered into the same collecting tube which contained Fraction II. This filtration required only about 10 minutes.

The residue on the filter cup, representing Fraction I + III, was removed as completely as possible with a small stainless steel spatula. Three small portions of Citrate Reagent were drawn through the filter in order to remove any protein remaining in the pores. These effluents were used to dissolve the paste previously removed with the spatula, and the whole was made up to 5 ml. with Citrate Reagent and taken for analysis. Allowing the suspension to warm slightly by exposing it to room temperature facilitated solution.[13]

Reprecipitation of the protein components of Fraction II was effected by adding 35 ml. of Sulfate Reagent to the Fraction II filtrate and washings. If for subsequent assays the presence of zinc ions was undesirable, and their removal or neutralization inconvenient, 35 ml. of Tartrate Reagent was substituted for the Sulfate Reagent.[14] In either case, the precipitate was allowed to form for one hour before removal by filtration in the cold bath through a glass filter cup with a maximum porosity of 14 microns. The filtration required about 15 minutes. The precipitated Fraction II was dissolved and made up to 5 ml. with distilled water or 0.15 M sodium chloride.

### *Analytical Methods*

*Nitrogen determinations* were carried out by the micro-Kjeldahl method. Non-protein nitrogen (NPN) was determined after precipitation of the proteins with 10% trichloracetic acid.

*Biuret determinations* were carried out by a modification of the method of Mehl (7) in which each series of readings was standardized against a standard preparation of normal human serum albumin ([6] footnote 14).[15] Determinations were made by diluting each aliquot to 10 ml. with 0.15 M sodium chloride before addition of 1

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occluded in Fraction I + II + III and appeared subsequently in Fraction II, resulting in contamination of the $\gamma$-globulins in Fraction II to the extent of about 20% with albumins. Carrying out the initial precipitation at higher dilutions did not circumvent this difficulty as satisfactorily as did the introduction of the washing step.

[13] A small amount of material often failed to dissolve.

[14] When the Tartrate Reagent was employed, precipitation of the proteins was only about 95% complete, but could be made quantitative by carrying out the filtration at −8°.

[15] Crystalline bovine serum albumin, obtainable from Armour and Co., Chicago, Ill., may be used in place of the human serum albumin without the need of applying any corrections due to the species difference.

ml. of the biuret reagent. The reagent was prepared as follows. To a solution of 20.5 g. cupric chloride dihydrate in 300 ml. of water, 150 ml. of ethylene glycol was added, followed by 300 g. of sodium hydroxide in aqueous solution, and the whole was diluted to 1 L. with water and transferred to a large Erlenmeyer flask covered with a watch-glass, and heated for four hours on a steam bath. The solution was filtered after cooling. Color development was allowed to proceed for 20 to 30 minutes before measurement in a Leitz photoelectric colorimeter set at 550 m$\mu$. (Klett and Coleman instruments have also been employed.)

*Lipid analyses* were carried out on ethanol-ether extracts prepared as follows. The protein solutions were added to about 20 volumes of ethanol-ether mixture (3:1 by volume), heated to boiling, cooled, and made up to 25 volumes. Aliquots were taken after filtration for the determination of total cholesterol and lipid phosphorus. *Total cholesterol* was determined by the method of Bloor, Pelkan and Allen (8). The photoelectric colorimeter was set at 640 m$\mu$. *Lipid phosphorus* was determined by the method of Fiske and SubbaRow (9) following the ashing procedure of Gortner (10). Color intensity was measured at 640 m$\mu$. The factor 25.0 was used to convert lipid phosphorus to phospholipid.

*Serum albumin determinations* were carried out by the hemin-binding method of Rosenfeld and Surgenor (11).

*Hexose determinations* were made according to a modification of the method of Sørensen and Haugaard (12) introduced by Friedmann (13). The reaction mixture contained 1 ml. of protein solution, 2.5 ml. of 1.6% orcinol in 50% sulfuric acid (by volume) and 15 ml. of 60% sulfuric acid. Color was developed by heating for 20 minutes at 80°, cooling in ice in the dark for 20 minutes, then standing at room temperature in the dark for 30 minutes. The color was measured at 445 m$\mu$. An equimolar mixture of galactose and mannose was used to prepare the standards included in each series of determinations.

*Fibrinogen determinations* (clottable protein) were made according to the method of Morrison (6).

*Determinations of pH* were carried out in the glass electrode at 25°, using 1 ml. of reagent solution or of protein suspension mixed with 4 ml. of 0.02 M sodium chloride.

*Electrophoretic analysis* of plasma and of the various fractions was used for supplementary characterization of the various fractions. The measurements were carried out in a sodium diethyl barbiturate-sodium citrate buffer at pH 8.6 and an ionic strength of 0.1 by the standard procedure employed in this laboratory (14). The buffer contained per L. 11.05 g. of barbital, 2.45 g. of sodium citrate ($2H_2O$) and 50 ml. of 1N NaOH.

*Determinations of dry weight,* as a method of total protein determination, were carried out as follows:[16]

A length of cellophane tubing 0.5 inch in diameter was weighed, soaked in water, and a knot was tied in one end. A known volume of the solution to be analyzed containing 100 to 300 mg. protein was added from a carefully wiped pipette introduced well down inside the bag. A small volume of distilled water was used to wash down any protein adhering near the opening of the bag. The bag was tied with sufficient slack to avoid squeezing the protein solution above the knot and to allow room for expansion on freezing. At the same time an equal length of tubing was tared and filled with an equal volume of distilled water. The two bags were dialyzed for three to four days against several daily changes of distilled water. The bags were then frozen rapidly by immersion in 95% ethanol cooled by a bath of dry ice in ethanol. The frozen bags were immediately transferred to a vacuum oven connected to a dry ice-ethanol trap and vacuum pump. The temperature of the oven was immediately raised to 75°. Constant weight was reached within 12 hours.

The initial weight of the tubing containing the unknown sample was corrected on the assumption that it underwent the same proportionate decrease in weight on drying as the control.

*Neutralization or removal of zinc or barium ions* added in performing the separations was found necessary for some assays. This was accomplished by one of the following methods. For many purposes it was found sufficient to neutralize the effect of the metals by the addition of sodium citrate or the more effective ethylenediaminetetraacetate[17] without actually removing the metals from the solution. When complete removal of the added cations was desirable, Fraction IV + V, or VI, was dissolved in water and passed through a cationic exchange resin column at 0°. A column of Dowex-50 exchange resin[18] 10 cm. in height and 2 cm. in diameter resulted in removal of usually more than 99% of the added divalent metals when the protein solution was allowed to pass through at the rate of 0.25 ml./cm.$^2$/min. Since it is necessary that the resin column be covered with liquid at all times, liberal quantities of distilled water must be passed through the column to displace the protein solution. When removal of the last traces of metals was not required, it was sufficient to stir the protein solution directly with the resin and to wash by decantation.

## *Analysis of Plasma and Separated Fractions*

The analytical procedures were applied to the various fractions as follows:

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[16] We are indebted to Dr. M. A. Lauffer, University of Pittsburgh, for the general description of this method.

[17] The trisodium salt of ethylenediaminetetraacetic acid was supplied as Sequestrene NA3 by the Alrose Chemical Co., Providence, R. I.

[18] The cationic exchange resin, Dowex-50 (plant grade, 30–50 mesh) may be obtained on the sodium cycle from National Aluminate Corp., Chicago 38, Ill., under the name Nalcite HCR. The only preparation necessary consisted in washing the resin by swirling in a flask with successive volumes of hot distilled water and allowing it to settle. This process was continued until the water above the settled resin was clear. The resin may be reactivated by treating for 15 minutes with 0.15 M sodium chloride, followed by thorough washing with distilled water.

*Plasma*

*a*) Total protein was calculated by applying the factor 6.73 to the value for protein nitrogen (15).[19] To correct the values obtained on the A.C.D. plasma (*i.e.*, plasma with acid citrate dextrose as anticoagulant) to absolute values, the nitrogen determination was repeated on plasma from heparinized blood drawn at the same time.[20] The factor (N in heparinized plasma/N in A.C.D. plasma) has been applied to all data for A.C.D. plasma and the various fractions.[21]

*b*) Total cholesterol and lipid phosphorus were determined on an aliquot of 2 ml.

*c*) Fibrinogen was determined on an aliquot of 1 ml.

*d*) Serum albumin was occasionally determined on an aliquot of 1 ml. made up to 125 ml. with sodium phosphate buffer of pH 7.3 and ionic strength 0.3.

*Fraction IV + V*

*a*) Total protein was determined by the biuret reaction on an aliquot of 0.5 ml.

*b*) Total cholesterol and lipid phosphorus were determined on an aliquot of 2 ml. The value obtained for total cholesterol was converted into the value for α-lipoproteins by multiplying by 6.25.[22]

*c*) Serum albumin was determined on an aliquot of 1 ml. made up to 100 ml. with sodium phosphate buffer of pH 7.3 and ionic strength 0.3.

*d*) Hexose was determined on an aliquot of 1 ml.

*Fraction VI*

*a*) Total protein was determined by the biuret reaction on an aliquot of 5 ml.

*b*) Hexose was determined on an aliquot of 2 ml.

*Fraction IV + V + VI*

Occasionally the solution of Fraction IV + V + VI was analyzed as a whole for total protein, total cholesterol, lipid phosphorus and serum albumin before Step No. 2 was carried out. The presence of the free carbohydrate (glucose) of plasma in Fraction IV + V + VI made impossible hexose determinations before separation of the glucose by washing Fraction VI.

*Fraction II*

*a*) Total protein was determined by the biuret reaction on an aliquot of 5 ml. of the extract of Fraction II before precipitation. Five ml. of Reagent B′ were included in the standard and blank biuret reagent mixtures to allow for the small enhancement of optical density due to the formation of copper-glycine complexes. Alternatively, 2 ml. of the redissolved Fraction II solution were used without the necessity of making allowance for the interference of glycine.

*b*) Total cholesterol and phospholipid were determined on an aliquot of 3 ml. of the redissolved Fraction II.

*Fraction I + III*

*a*) Total protein was determined by the biuret reaction on an aliquot of 1 ml.

*b*) Total cholesterol and lipid phosphorus were determined on an aliquot of 2 ml. The value obtained for total cholesterol was converted into the value for β-lipoproteins by multiplying by 2.86.[23]

For the calculation of the total protein in Fraction I + III, the value for the biuret determination was corrected by the addition of the quantity (g. per 100 ml. of cholesterol) × 2.14, based on the presence of 75% total lipids in the β-lipoproteins (17). On the average such a correction gave a ratio of total protein to biuret value of 1.27. This calculation may be expected to fail when applied to the fraction separated from lipemic plasma or plasma containing highly atypical lipoproteins. The necessity for including the lipid correction was shown by comparison of the value for total protein obtained by dry weight with the biuret value. For a typical Fraction I + III, the ratio of dry weight to biuret value was 1.19, in contrast to ratios of 1.02 and 0.97 for Fractions IV + V and II, respectively. Because of the small deviations, it

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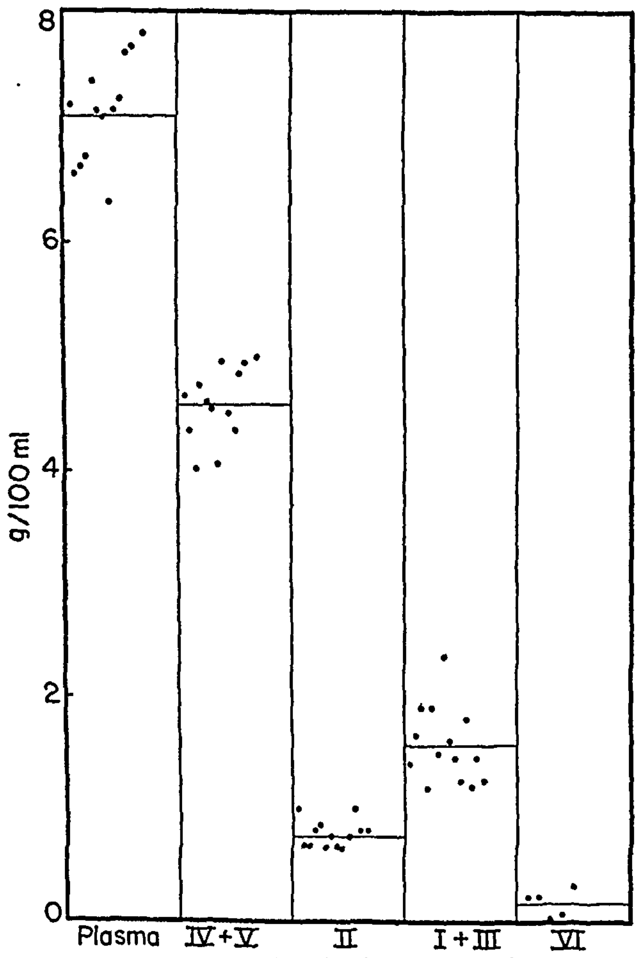
[19] In the usage of this Laboratory, "total protein" is synonymous with "total non-dialyzable solids." This practice allows a more direct interpretation of electrophoretic and ultracentrifugal data than the use of some purely arbitrary value such as N × 6.25, since the contribution of the lipids to the refractive index of plasma or of the fractions is only a little less than that of a corresponding weight of protein (14). The theoretical justification of such a practice is that in normal fasting plasma all, or almost all, of the lipids appear to be present in the form of well-defined lipoproteins, and tally should be kept of the whole of such components rather than only of their peptide part (16, 17). The same argument applies to protein-bound sugar. The factor 6.73 is most accurately employed with pooled normal plasma but obviously will not, in general, be correct for highly abnormal plasmas. For this latter purpose, individual determinations of dry weight are recommended.

[20] The use of heparin as anticoagulant for the plasma to be fractionated was avoided on the advice of Dr. Alfred Chanutin who had observed that in such a case Fraction I + II + III tended to clot readily. The possibility that heparin would affect the solubility behavior of various plasma proteins was also a deterrent to its use.

[21] The value of this factor varied between 1.10 and 1.30, with an average of about 1.20. Comparison with the value of 1.32 which would be predicted (for a plasmacrit of 0.55) on the basis of simple dilution with the acid citrate dextrose solution shows a varying effect of the A.C.D. solution on the red cell volume.

[22] For this calculation the percentage of cholesterol in the α-lipoproteins was taken as 16 ([3] Table VIII, Fraction IV-1, 1).

[23] For this calculation the percentage of cholesterol in the β-lipoproteins was taken as 35, since it was found that the cholesterol determination employed in this procedure yielded 35% cholesterol instead of the average of 30.9% obtained with a more accurate procedure (16).



| % Total Plasma Proteins | IV+V | II | I+III | VI |
|---|---|---|---|---|
| Average | 65.6 | 10.8 | 22.0 | 2.2 |
| High | 71.7 | 14.0 | 32.0 | 3.3 |
| Low | 59.8 | 9.3 | 15.8 | 0.2 |

FIG. 2. CONCENTRATION OF TOTAL PROTEIN IN PLASMA AND IN THE FOUR PRIMARY FRACTIONS

has been considered unnecessary for routine purposes to apply corrections to the total protein values obtained by the biuret determinations on Fraction IV + V and II.

## RESULTS

### *Protein Distribution*

Representative data for the concentration of total protein in plasma and in each fraction are given in Figure 2. The average for each group of data is indicated by a horizontal line. Average and extreme high and low values for the per cent of the total plasma proteins in each fraction are shown at the bottom of the figure.

### *Cholesterol and Phospholipid Distribution*

The distributions of cholesterol and phospholipid among the fractions are shown in Figure 3. The mole ratio of cholesterol to lipid phosphorus among the fractions is presented in Table I. The quantities of cholesterol and phospholipid separated in each fraction were slightly more variable than the total protein (Figure 2); yet the constancy of the distribution was still striking. Perhaps most interesting was the finding of the fairly constant and yet widely differing mole ratios of cholesterol to lipid phosphorus in Fractions IV + V and I + III, associated with α-lipoproteins and β-lipoproteins respectively (Table I).[24] Fraction IV + V, containing the α-lipoproteins, had significantly larger amounts of phospholipids than of cholesterol; whereas in Fraction I + III, containing the β-lipoproteins, the amount of cholesterol was greater than that of phospholipids. Some of the other contrasting properties of these two groups of lipoproteins have been described elsewhere (17). The high ratio of cholesterol to phospholipid found in Fraction II is characteristic of neither of the main groups of α-lipoproteins or β-lipoproteins, and invites further study to establish the form in which these lipids are present in Fraction II.

The proportion of the total cholesterol in the esterified form was determined on the fractions of six samples of plasma by the method of Schoenheimer and Sperry (18). The average values for the per cent esterified cholesterol were 73.4%, 70.1% and 76.7% for plasma, Fraction I + III, and Fraction IV + V, respectively. These results confirm the essential constancy of the re-

TABLE I

*Mole ratio of total cholesterol to lipid phosphorus in plasma fractions*
Mole ratio cholesterol/lipid P

| | Fraction | | | Plasma |
|---|---|---|---|---|
| | I + III | II | IV + V | |
| | 2.21 | 5.75 | 1.01 | 1.52 |
| | 2.33 | 4.10 | 0.72 | 1.63 |
| | 2.50 | 3.88 | 0.80 | 1.70 |
| | 2.31 | 3.90 | 0.72 | 1.72 |
| | 2.18 | 5.88 | 0.78 | 1.88 |
| Average | 2.31 | 4.70 | 0.81 | 1.69 |

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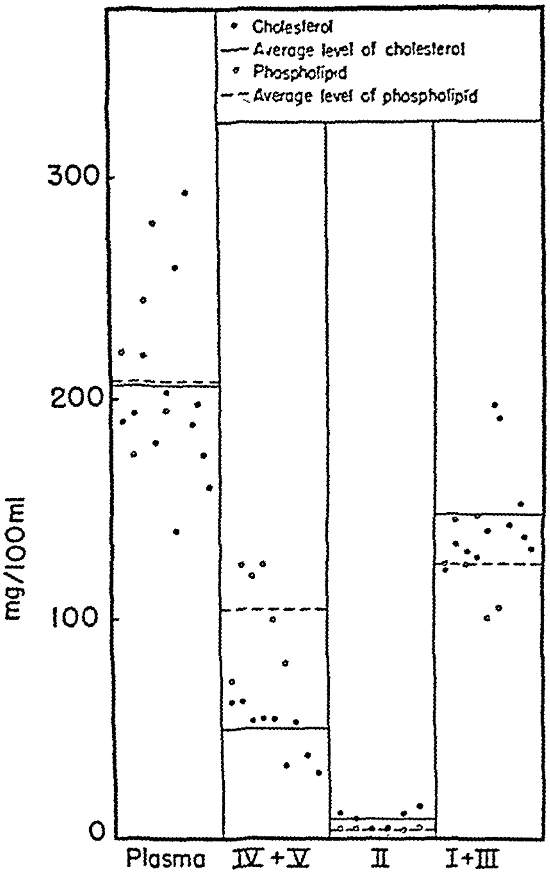
[24] The mole ratio in Fraction I + III, 2.31, is in agreement with the value of 2.11 calculated from the data of Oncley, Gurd and Melin (16) for the preparations of purified β-lipoproteins separated from large pools of normal plasma. The present values would have been slightly lower if the more tedious analytical procedure (involving hydrolysis of cholesterol esters) used by Oncley and his associates had been employed.



| | % Total Cholesterol | | |
|---|---|---|---|
| Average | 26.6 | 4.0 | 71.4 |
| High | 33.3 | 6.9 | 83.1 |
| Low | 18.0 | 1.5 | 54.8 |
| | **% Total Phospholipid** | | |
| Average | 46.7 | 1.5 | 52.8 |
| High | 57.0 | 2.3 | 56.6 |
| Low | 33.0 | 0.8 | 51.0 |

FIG. 3. DISTRIBUTION OF CHOLESTEROL AND PHOSPHOLIPID AMONG THE FRACTIONS

lationship between esterified and unesterified cholesterol in the major lipoprotein fractions, first shown in fractions prepared by a forerunner of the present method (3).[25]

Fraction VI was found to contain at most immeasurably small traces of cholesterol and phospholipid.

### *Carbohydrate Distribution*

The distribution of protein-bound carbohydrate (hexose) among the protein fractions is presented in Figure 4. The lack of information about the

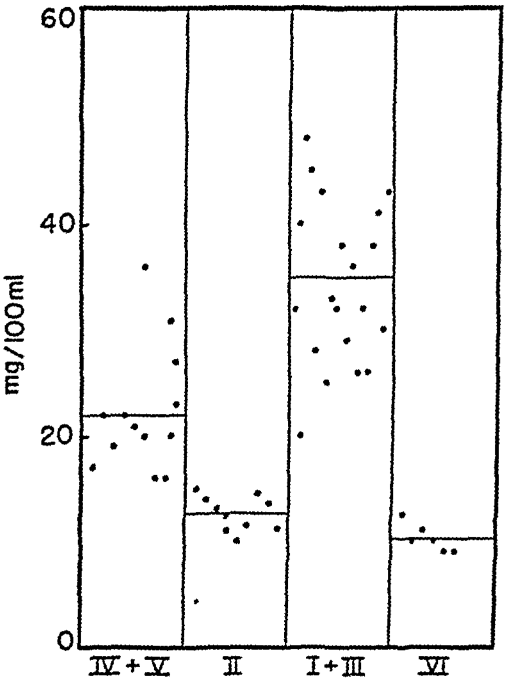
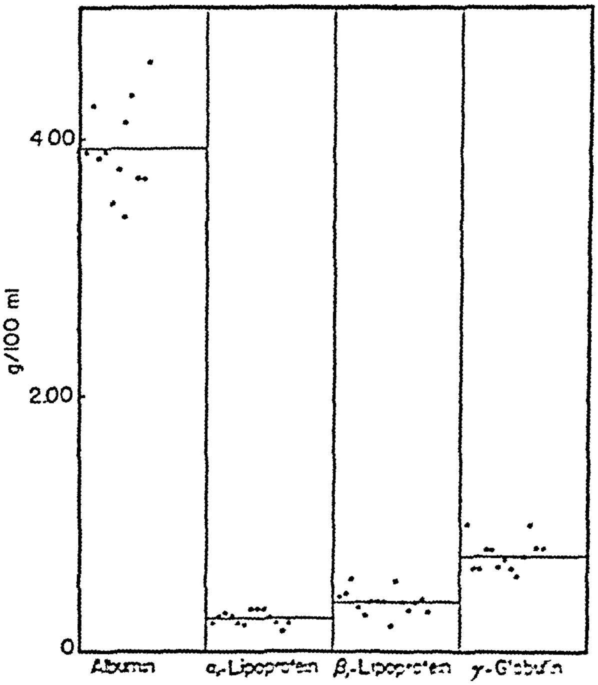


FIG. 4. DISTRIBUTION OF PROTEIN-BOUND CARBOHYDRATE (HEXOSE) AMONG THE FRACTIONS



| | % Total Plasma Protein | | | |
|---|---|---|---|---|
| Average | 56.5 | 3.8 | 5.5 | 10.8 |
| High | 65.5 | 6.0 | 8.6 | 14.0 |
| Low | 51.1 | 2.0 | 3.1 | 9.3 |

FIG. 5. CONCENTRATIONS OF SERUM ALBUMINS, α-LIPOPROTEINS, β-LIPOPROTEINS AND γ-GLOBULINS IN PLASMA DETERMINED BY CHEMICAL ANALYSIS OF THE FRACTIONS

exact quantities of hexose present in the complicated group of glycoproteins and mucoproteins of normal human plasma, coupled with the rather general distribution of protein-bound carbohydrate throughout the globulins in plasma (20), has made it impossible to interpret these data in a way similar to the lipid distributions (Figure 5). The data are presented as a useful definition of normal hexose distribution. The hexose in Fraction VI, normally combined principally with an $\alpha_1$-globulin described by Schmid (21), has been found to show marked increases in certain abnormal plasmas.

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[25] Personal communications from Drs W. M. Sperry, M. Melin, and A. Chanutin. For more recent studies, see Pearsall and Chanutin (19).

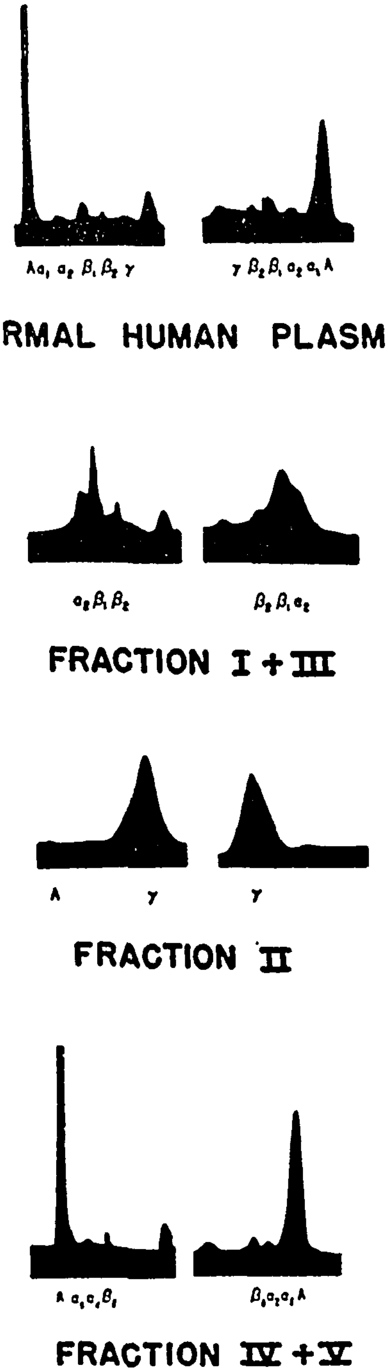

FIG. 6. ELECTROPHORETIC DIAGRAMS OF HUMAN PLASMA AND OF THREE OF THE FOUR PRIMARY FRACTIONS

TABLE II
*Distribution of electrophoretic components in Fraction IV + V*

| | Per cent of fraction | | | | | | |
|---|---|---|---|---|---|---|---|
| | Albumin | $\alpha_1$ | $\alpha_2$ | $\beta_1$ | $\beta_2$ | Fibrinogen | $\gamma$ |
| | 85 | 7 | 2 | 5.5 | 0.5 | — | — |
| | 81 | 6 | 6 | 7 | — | — | — |
| | 80 | 10 | 4.5 | 5 | 0.5 | — | — |
| | 81 | 6 | 7 | 5 | 1 | — | — |
| | 84 | 5 | 4.5 | 6 | 0.5 | — | — |
| | 84 | 7 | 3 | 5 | 1 | — | — |
| | 82 | 6 | 5 | 6 | 1 | — | — |
| | 79 | 10 | 4 | 6 | 1 | — | — |
| | 84 | 7 | 3 | 6 | — | — | — |
| | 78 | 12 | 3.5 | 6 | 0.5 | — | — |
| | 79 | 8 | 9 | 3 | 1 | — | — |
| | 78 | 11 | 5 | 5 | 1 | — | — |
| Average | 81.3 | 7.8 | 4.7 | 5.5 | 0.7 | — | — |

## *Electrophoretic Composition of Fractions*

Typical electrophoretic composition data for Fractions IV + V, II, and I + III are presented in Figure 6 and in Tables II, III, and IV, respectively. The values for $\beta_2$-globulins in Table II are on the borderline of interpretation, and could have been included with the $\beta_1$-peak in the diagrams. Fraction IV + V separated on larger scales (2) was very rarely found to contain $\beta_2$-globulins.

## *Concentration of Serum Albumins, α-Lipoproteins, β-Lipoproteins, γ-Globulins and Fibrinogen*

The concentrations of serum albumins, $\alpha$-lipoproteins, $\beta$-lipoproteins, and $\gamma$-globulins are shown

TABLE III
*Distribution of electrophoretic components in Fraction II*

| | Per cent of fraction | | | | | | |
|---|---|---|---|---|---|---|---|
| | Albumin | $\alpha_1$ | $\alpha_2$ | $\beta_1$ | $\beta_2$ | Fibrinogen | $\gamma$ |
| | 6 | — | — | — | 4 | — | 90 |
| | 4 | — | — | — | 4 | — | 92 |
| | 4 | — | — | — | 4 | — | 92 |
| | 3 | — | — | — | 2 | — | 95 |
| | 5 | — | — | — | 3 | — | 92 |
| | — | — | — | — | 4 | — | 96 |
| | 1 | — | — | — | 3 | — | 96 |
| | 1 | — | — | — | 1 | — | 98 |
| | 2 | 1 | — | — | 5 | — | 92 |
| | 5 | — | — | — | 8 | — | 87 |
| Average | 3.1 | 0.1 | — | — | 3.8 | — | 93.0 |

in Figure 5, along with the percentages of the total plasma proteins which they represent. Serum albumins were determined by the hemin-binding method on Fraction IV + V rather than on whole plasma in order to avoid errors due to any nonspecific interference of other proteins.

The quantities of α-lipoproteins and β-lipoproteins accord well with previous estimates (22, 16) based on fractionation studies of large pools of normal human plasma, and with the studies on individual sera reported by Gofman, Lindgren and Elliott (23).

In view of the rather satisfactory electrophoretic (Figure 6, Table III) purity of the γ-globulins in Fraction II, the total protein in Fraction II has been taken for the present purposes as a measure of the total γ-globulins of plasma. Data to be presented below concerning the distribution of added γ-globulin, along with considerable experience with pathological plasmas in which the γ-globulins were elevated, have confirmed the justification for this approximation.

TABLE IV

*Distribution of electrophoretic components in Fraction I+III*

| | Per cent of fraction | | | | | |
|---|---|---|---|---|---|---|
| | Albumin | $\alpha_1$ | $\alpha_2$ | $\beta_1$ | $\beta_2$ + Fibrinogen | $\gamma$ |
| | — | — | 13 | 53 | 27 | 7 |
| | — | 3 | 9 | 55 | 23 | 10 |
| | — | 4 | 10 | 50 | 29 | 7 |
| | 1 | 1 | 11 | 57 | 28 | 2 |
| | — | 3 | 14 | 45 | 31 | 7 |
| | — | 3 | 10 | 58 | 26 | 3 |
| | — | 5 | 57 | | 32 | 6 |
| | — | 5 | 54 | | 36 | 5 |
| | 2 | 4 | 24 | 43 | 19 | 8 |
| | 1 | 4 | 60 | | 31 | 4 |
| | 1 | 6 | 29 | 30 | 28 | 6 |
| | 2 | 2 | 14 | 50 | 23 | 9 |
| | — | 5 | 8 | 55 | 21 | 11 |
| | — | 2 | 9 | 61 | 18 | 10 |
| Average | 0.5 | 3.5 | 13.3 | 49.4 | 26.5 | 6.8 |

In some of the early studies, fibrinogen was determined on Fraction I + III by the method employed by Morrison for the study of Fraction I (6). These determinations were discontinued because of extensive occlusion in the clot of the other proteins present in Fraction I + III, in confirmation of the findings of Morrison with Fraction III-0 euglobulin, a fraction also rich in β-lipoproteins (24).[26] From previous studies on citrated plasma in this laboratory (25), using the method of Cullen and Van Slyke (26), a corrected average value of 0.28 g. fibrinogen per 100 ml., or 4.04% of the total plasma proteins may be taken to supplement the data of the present series.

### *Recovery of Added Plasma Proteins*

The recoveries in the separated fraction of added γ-globulins, β-metal combining protein and β-lipoproteins were studied. The γ-globulin preparation employed was prepared on a large scale by Method 10 (2) and was 99% pure by electrophoresis, with albumin as the only impurity detected. The crystalline β-metal combining protein (27) and the purified β-lipoproteins (16) were isolated as previously described. Each protein was added in concentrated solution to one half of a sample of plasma so as to raise the concentration of the constituent to roughly twice the normal, leaving the other half as the control. Table V shows the increase in the various fractions calculated as per cent of the quantity of the constituent added.

The distribution of the added γ-globulins between Fractions II and I + III was as favorable as for normal concentrations. By electrophoretic analysis, γ-globulins remained absent from Fraction IV + V, and, as shown in Table VI, no significant changes occurred in the electrophoretic distribution of Fraction I + III.

TABLE V

*Recovery of added plasma proteins*

| Protein added | | Increase in fraction as per cent added constituent | | |
|---|---|---|---|---|
| Nature | Conc. | IV + V | II | I + III |
| | *g./100 ml.* | | | |
| γ-globulins | 0.60 | 0 | 93 | 10 |
| β-metal combining protein | 0.25 | 0 | 0 | 0 |
| β-lipoproteins | 0.39 | 0 | 0 | 92 |
| (as cholesterol) | 0.13 | 2 | −1 | 93 |

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[26] A number of determinations of fibrinogen were made on separate samples of the plasma by the method of Morrison (6). The data were corrected on the assumption, based on an extrapolation of the data of Morrison, that occlusion of other proteins amounted to 40% of the weight of the fibrin. The range of values so obtained was 0.15 to 0.43 g. per 100 ml., with an average of 0.30 g. or 4.3% of the total plasma proteins.

TABLE VI

*Effect of addition of plasma proteins on electrophoretic composition of fractions*

| Protein added | Fraction analyzed | Per cent of fractions | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Albumin | $\alpha_1$ | $\alpha_2$ | $\beta_1$ | $\beta_2$ + Fib. | $\gamma$ |
| $\gamma$-globulins | I+III | Before | 1 | 7 | 64 | | 25 | 3 |
| | | After | 4 | 6 | 56 | | 29 | 4 |
| $\beta$-metal comb. protein | IV+V | Before | 83 | 9 | 3 | 5 | — | — |
| | | After | 78 | 8 | 4 | 9 | — | — |
| $\beta$-lipoproteins | I+III | Before | — | 6 | 58 | | 30 | 6 |
| | | After | — | 5 | 66 | | 21 | 8 |

The $\beta$-metal combining protein, due to the small amount added, failed to cause a change in the gross distribution of the proteins among the fractions (Table V). However, the electrophoretic data showed clearly the marked increase in $\beta_1$-globulins in Fraction IV + V (Table VI), whereas no changes were observed in the other fractions.

The recovery of $\beta$-lipoproteins in Fraction I + III, and the failure to contaminate the $\alpha$-lipoproteins in Fraction IV + V, were striking. Table VI shows the absence of any significant increase in the small quantity of $\gamma$-globulins present in Fraction I + III.

These recovery experiments confirmed the selectivity of the separations, and showed that considerable increases in at least certain of the plasma proteins did not derange the system of fractionation. It was particularly important that large variations in the proportions of the strongly interacting components, $\gamma$-globulins and $\beta$-lipoproteins, could be tolerated in the separation of Fraction I + II + III and the subsequent extraction of Fraction II.

## SUMMARY

1. Method 10 of plasma fractionation developed by Cohn and his co-workers has been adapted for the quantitative estimation of several protein and lipoprotein components in small quantities of normal human plasma.

2. In the system of analysis presented in this communication samples of 5 ml. of plasma were separated by filtration into four fractions and the amounts of albumin, $\gamma$-globulins, $\alpha$-lipoproteins and $\beta$-lipoproteins were determined. In addition, the distributions of cholesterol and phospholipid and of protein-bound carbohydrate (hexose) among the fractions were studied.

3. The proteins are present in the various fractions in an unaltered state. Their biologic activities are unimpaired and can be measured.

4. Of interest is the concentration in Fraction II of more than 90% of the $\gamma$-globulins of the plasma in a purity exceeding 90% by electrophoretic measurements. The other three fractions contain mixtures of unrelated proteins.

5. The plasma lipoproteins are divided into two primary groups; the $\alpha$-lipoproteins in Fraction IV + V contain a considerably higher proportion of phospholipids to cholesterol than the $\beta$-lipoproteins in Fraction I + III.

6. Added amounts of various pure proteins were in each case recovered in the appropriate fractions, proving that even large variations in the proportions of the various proteins do not interfere with the system of fractionation.

## ACKNOWLEDGMENT

The authors wish to express their deep appreciation to Dr. Edwin J. Cohn for suggesting this investigation and for his advice and constructive criticism.

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# PROTEOLYTIC ENZYMES AND PLATELETS IN RELATION TO BLOOD COAGULATION[1]

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(Submitted for publication August 11, 1950; accepted, October 30, 1950)

Ferguson's (1, 2) concept of a role for proteolytic enzymes (3) in the mechanisms of blood clotting was that of a weak digestive action "disaggregating" (Pope, 1938) the complex unions of plasma proteins with calcium and thromboplastic phospholipid (probably cephalin), thereby mobilizing or making available these activators and thus, in a sense, "catalyzing" the prothrombin activation (4). Observations believed to support such a theory included: *1*) the clot-aiding effects, particularly referable to the conversion of prothrombin into active thrombin, on adding (crystalline) trypsin (2, 4); and *2*) the evidence, not altogether conclusive (4) nor confirmed (5–7), that preparations of serum protease (tryptase, plasmin, or fibrinolysin) resemble pancreatic trypsin in many ways although differing significantly in others (3).

The establishment of crucial data in this, as in all work on the problem of the conversion of prothrombin to thrombin, is technically difficult because of the biochemical complexities, the presence in the natural clotting system (*e.g.* plasma) of unrecognized factors (some of which have recently been revealed), and questions as to contaminants (the known ones being demonstrable) in even the purest of the isolated reagents available to date (4). From the start (1939) of our work with the enzyme problem we have accepted the current, and still valid, working hypothesis that, under all ordinary ("physiological ") circumstances, prothrombin requires for its activation free Ca-ions and a phospholipid-containing material for which the terms "thromboplastin" or "thrombokinase" are commonly used. It has more recently been shown by a number of workers that other factors are also involved and evidently must have been unwittingly provided in the test materials of all early experiments. Our own observations (8) that, with very carefully purified and treated reagents, no thrombin could be detected in over 24 hours in mixtures of prothrombin, brain thromboplastin, and calcium salt, but required the addition of another factor or factors which could be supplied in the form of Ware and Seegers' (9) "serum accelerator globulin" (AcG) preparation, caused us to add this reagent to the purified test systems in the present studies. Under these circumstances, as in the past, we feel that our studies are concerned primarily with the basic "thromboplastic" mechanisms.

The present work commences with the confirmation of the clot-aiding effects of trypsin and of serum fibrinolysin on ordinary plasma and supplements this with an experiment on platelet-free plasma. Proceeding thence to the study of thrombin formation from highly purified prothrombin (with added AcG), a number of enzyme preparations, activators, and inhibitors have been tested, in various combinations, in an effort to shed further light upon the possible mechanisms of action of the proteolytic enzymes in such clotting systems. The thromboplastic role of platelets in relation to these phenomena has been given special attention. The weight of evidence eventually leads to the conclusion that whereas trypsin does seem to act with parallelism of its clot-aiding and proteolytic effects, serum fibrinolysin can still aid thrombin formation (in these systems) even when the proteolytic action is reduced greatly or abolished by appropriate enzyme inhibitors. This must suggest another factor in the serum enzyme preparation, the possible nature and mode of action of which are investigated and discussed.

## MATERIALS

1. Borate *buffer* solution, pH = 7.75, specific resistance 170 ohms (at 21°C), effective ionic strength 0.055, contained (per liter): 11.25 gm. $H_3BO_3$, 4 gm. $Na_2B_4O_7$, 12 $H_2O$, 2.25 gm. NaCl.
2. *Plasma*, oxalated or citrated, was prepared from dog or beef blood. In special studies, cited in the text,

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[1] This investigation was supported by a research grant from the Division of Research Grants and Fellowships of the National Institutes of Health, U. S. Public Health Service.

(*a*) "deplateletized" plasma (PFP) was prepared by the silicone technic (10) and long centrifugation (multispeed) in the refrigerated centrifuge; (*b*) "defibrinated" plasma (FFP) was obtained by heating at 51°C for one-half hour and centrifugation; (*c*) "prothrombin-free" plasma (S.Pl.) was obtained by five successive filtrations through Seitz pads, according to the method of Owren (11).

3. *Serum,* obtained from beef blood, was rendered prothrombin-free by adsorption with $BaSO_4$ (12).
4. *Fibrinogen:* ($F_1$) 0.5–1.0% solution in borate buffer of Armour's bovine fibrinogen (Fraction I). This was free from fibrinolysin but contained small traces of prothrombin which, for many tests, was removed by $BaSO_4$ adsorption ($F_2$).
5. Purified *prothrombin* (Pro), bovine, preparations of Dr. W. H. Seegers (Wayne University), stock solution (0.1%) in borate buffer. The solutions used in *most of the cited experiments contained an insignificant* trace of thrombin (clotting times with $F_2 > 30$ min.) and some "accelerator" factor(s), but the latter was always supplemented with added AcG.
6. *Accelerator globulin* (AcG), bovine "serum type," preparation of Drs. A. G. Ware and W. H. Seegers, stock solution (0.1%) in borate buffer. It contained traces of prothrombin and a minute amount of thrombin, considered non-significant in the dilutions employed for the cited experiments.
7. *Thromboplastins:* Tpln. A, a highly active thromboplastin prepared from acetone-dried dog brain; Tpln. B, Schieffelin's stabilized (horse brain) thromboplastin, courtesy Drs. E. W. Blanchard and C. F. Gerber.
8. *Fibrinolysin* (Lysin): purified preparation from beef serum, courtesy Dr. E. C. Loomis (Parke Davis Co., Detroit). 1% stock solution freshly prepared immediately before use.
9. *Antifibrinolysin* (AFln): purified bovine preparation, courtesy Dr. Loomis.
10. *Trypsin* (pancreatic): (Tryp.), commercial preparation (Fairchild Bros., Foster, N. Y.); *Crystalline trypsin* (XT), courtesy Dr. M. Kunitz (Rockefeller Institute, Princeton).
11. *Soybean inhibitor* (antiprotease): crystalline preparation (SBI), courtesy Dr. Kunitz.
12. *Pancreatic inhibitor* (antiprotease) (PI): Sharp and Dohme (Lot P. A–97–1), courtesy Dr. L. A. Kazal.
13. *Washed platelets* (Plat.): 100 ml. dog blood collected into 13 ml. 3.4% sod. citrate. Repeated centrifuging for a few minutes at 1000 r.p.m. until red cells no longer visible in sediment. Platelets then recovered by one-half hour centrifuging at 5000 r.p.m., resuspended and well washed three times by thorough mixing with successive 50 ml. saline (containing ⅕ vol. 3.4% citrate) and a fourth washing in plain 0.9% NaCl. Final sediment suspended in 1 ml. saline gave a concentrated preparation, Wright-stained smears of which showed only granular platelet material with no leukocytes and only a rare erythrocyte. Grinding the platelet sediment with distilled water on a number of occasions did not modify its behavior in our test systems. The thoroughness of the washing is believed satisfactory for the virtual absence of plasma contaminants.
14. *Thrombin* (Thr.): bovine thrombin (Upjohn's), courtesy Dr. J. T. Correll. Stock solution in borate buffer (100 u./ml.), diluted as required. For the tests of Table VII a special preparation was made and used as described in the text.

## *Effects of Fibrinolysin on Clotting of Recalcified Plasma*

In numerous experiments on oxalated plasma (dog: beef), optimally recalcified, Loomis' fibrinolysin consistently reduced the clotting-times, *e.g.* beef plasma (26°C): *1*) control, 330 sec.; *2*) *shorter clotting-times, with increasing lysin, to 135* sec. "optimum" at 1.5–2.0 mg./ml. lysin; *3*) lessening effects with further amounts of enzyme, *e.g.* 205 sec. at 6.0 mg./ml. In the last test, the clot lysed in one-half hour, whereas at 1.5–2.0 mg./ml. (optimal for clotting), fibrinolysis was incomplete in 48 hours at room temperature. That the inhibitory effect of plasma antilysin is responsible is evident from clot-lysis times for any single concentration of lysin tested in *a*) plasma clots, *b*) pure fibrin clots—see Table I.

## *Effects of Proteases, etc. on Clotting of Recalcified* Deplateletized *Plasma*

*Deplateletized* citrated dog plasma (PFP: see Materials) was used for the tests of Table I. Triplicate clotting tests immediately after preparation of PFP, using 0.9% NaCl diluent and simple recalcification at room temperature, showed no clotting until 32–36 hours. The marked clot-accelerating effects of 4 mg. Loomis' fibrinolysin, of *25–100* μg. crystalline trypsin, and of a diluted (suboptimal) brain thromboplastin, are evident. Five-tenths mg. soybean inhibitor reverses the clot acceleration in all cases, but particularly in the *case of trypsin.*

In these and other experiments we have made with deplateletized plasmas, clotting in times exceeding half an hour appeared to involve some unexplained questions of non-specific factors relating to colloidal stability, rather than specific ("thromboplastic") factors, like those in the data of Table I, which have a very marked influence in reducing the clotting time.

As in all plasma experiments, fibrinolytic effects are weak because of natural antilysin and prob-

TABLE I

*Effects of proteases, etc. on clotting times of platelet-poor plasma*

Clotting times and lysis times, at 38°C, of 0.5 ml. citrated dog plasma (platelet-poor: silicone technic), recalcified with 0.1 ml.M/10 $CaCl_2$, in presence of 1.0 ml. additional volume of borate buffer (pH = 7.75) containing the amounts of other reagents noted. ∞ = no lysis in 10 days.

| | Inhibitor | Activator | Clotting time | Lysis-time | |
|---|---|---|---|---|---|
| | | | | Plasma clot | Std. fibrin clot (13) |
| 1. | 0 | 0 | 2000 sec. | ∞ | |
| 2. | SBI (0.5 mg.) | 0 | 2880 sec. | ∞ | |
| 3. | 0 | Lysin (4 mg.) | 127 sec. | 5 days (tr.) | 5¾ min. |
| 4. | SBI (0.5 mg.) | Lysin (4 mg.) | 525 sec. | ∞ | 1½–2 days |
| 5. | 0 | XT (tryp.) (25μg.) | 365 sec. | 4–5 days | 9 min. |
| 6. | SBI (0.5 mg.) | XT (tryp.) (25μg.) | 2490 sec. | ∞ | 5 days |
| 7. | 0 | XT (tryp.) (50μg.) | 168 sec. | 2–3 days | 5½ min. |
| 8. | SBI (0.5 mg.) | XT (tryp.) (50μg.) | 2400 sec. | ∞ | 5 days |
| 9. | 0 | XT (tryp.) (100μg.) | 118 sec. | 18 hours | 3 min. |
| 10. | SBI (0.5 mg.) | XT (tryp.) (100μg.) | 1800 sec. | ∞ | 4½ days |
| 11. | 0 | Tpln. B (dil.) | 65 sec. | ∞ | |
| 12. | SBI (0.5 mg.) | Tpln. B (dil.) | 352 sec. | ∞ | |

ably also, in part, due to instability of the enzyme preparations over the long periods (days). The standard clot (fibrin) lysis times included in the last column of Table I afford a reliable measure of respective lytic potencies (13).

### *Effect of Fibrinolysin on Conversion to Thrombin of Prothrombin in Fibrinogen-Free Plasma*

In several experiments, beef plasma heated to 51°C for one-half hour and centrifuged from the coagulated protein was obtained fibrinogen-free, by tests with purified thrombin. Table II shows, by means of the shortening clotting times of successive samples removed after the stated incubation periods and tested on fibrinogen, the progressive thrombin formation in such fibrinogen-free plasma after recalcification, with and without fibrinolysin added. The evident shortening of the time required to reach the best (*i.e.* quickest) clotting time and the fact that this is faster (12 sec.) with the lysin present than in the control (21 sec.), show that the enzyme acts by facilitating thrombin formation. Any tissue thromboplastin will give similar results.

### *Effects of Fibrinolysin and Other Activators on Thrombin Formation from Purified Prothrombin*

Table III shows, by means of clotting times (sec.) of samples of various thrombic mixtures added to test fibrinogen after the stated incubation periods, the *relative* amounts of thrombin formation from a purified prothrombin solution. We have presented a critical analysis of this method in a recent publication (4). In brief, the shortest clotting time reached measures "complete" conversion of prothrombin to thrombin under the particular test conditions, while the incubation

TABLE II

*"Thromboplastic" action of fibrinolysin in activation of prothrombin in recalcified fibrinogen-free plasma*

Two and five-tenths ml. plasma (beef: defibrinated at 51°C for one-half hr.), recalcified with 0.25 ml. M/10 $CaCl_2$, with (*1*) 0.75 ml. borate buffer (control) or (*2*) 0.75 ml. 1% lysin. After stated incubation periods, 0.25 ml. samples tested with 0.5 ml. 0.5% fibrinogen ($F_1$), noting clotting times (sec.), at 26°C, pH = 7.75.

| | Added agent | Incubation period (*min.*) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1′ | 2′ | 3′ | 4′ | 5′ | 6′ | 7′ | 8′ | 10′ | 11′ | 15′ | 20′ |
| 1. | Buffer (only) | 200″ | 190″ | 150″ | 107″ | 60″ | — | 28″ | 24″ | 21″ | — | 21″ | 24″ |
| 2. | Lysin | 100″ | 50″ | 19″ | 14″ | 12″ | 12″ | — | — | — | 12″ | — | — |

period needed for this is a measure of the effectiveness of the activator factors. Since calcium is optimal and a sufficiency of other known accelerators is supplied in the AcG added (perhaps presumptively), the "thromboplastic" factor is believed to be the significant variable. We have not included in the present work any attempt to define a thrombin "unit" or "percentage" (of prothrombin activated), since mere inspection of the data in each experiment suffices clearly to bring out the essential points of comparison. Thus, in the experiments of Table III: *1*) No clots in one-half hour shows inadequacy of calcium and AcG alone to activate the purified prothrombin solution; *2*) fibrinolysin, in addition, has so little effect as to be practically negligible; *3*) platelets, on the other hand, contribute a definite "thromboplastic" effect and result in a fairly satisfactory thrombin formation, although inferior to a typical potent tissue thromboplastin (tests 5). The fact that lysin and platelets, acting in conjunction (tests 4), produce the best activation, though not yielding more thrombin (10 sec. clotting time optimum) than in tests 5, argues strongly for a "platelet thromboplastin potentiating" (PTP) action of the enzyme.

TABLE III

*Effects of fibrinolysin and other activators on thrombin formation from purified prothrombin*

Clotting times (sec.), at 26°C, pH = 7.75 (borate buffer), for 0.5 ml. $F_1$ + 0.25 ml. thrombic mixture (after stated incubation periods), containing 0.2 ml. Pro. (0.1%), 0.1 ml. AcG (0.1%), 0.5 ml. M/20 $CaCl_2$, and other activators in amounts specified, with borate buffer solution to total volume 5 ml. ∞ = No clot in one-half hr.

| | Activators | Incubation period (*min.*) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 1′ | 3′ | 5′ | 10′ | 20′ | 40′ | 60′ |
| 1. | 0 | — | — | ∞ | ∞ | ∞ | ∞ | ∞ |
| 2. | Lysin (3 mg.) | — | — | 390″ | 380″ | 368″ | 335″ | 320″ |
| 3. | Plat. (0.5 ml.) | 420″ | 155″ | 60″ | 21″ | — | — | 18″ |
| 4. | Lysin (3 mg.)<br>Plat. (0.5 ml.) | 75″ | 22″ | 16″ | 11″ | 11″ | — | 10″ |
| 5. | Tpln. A (0.5 ml.) | 240″ | 120″ | 10″ | 10″ | 10″ | 10″ | 10″ |

### *Clot-Aiding Effects of Platelets*

The designation of the mode of action of platelets as "thromboplastic" in the preceding section would not only be in line with the definition and working hypothesis presented in the introduction, but the presence of added AcG (ineffective, with Ca alone) would argue against the platelet activators being significantly of the type supplied by serum accelerator globulin. Since a paper by Ware, Fahey and Seegers (14), appearing while the present studies were in progress, claimed that platelets supplied very little thromboplastin, but significant amounts of AcG, and some factor (probably of the type we call "fibrinoplastic" [15]) which aids the thrombin-fibrinogen reaction, these claims were carefully and repeatedly investigated with our platelet preparations.

TABLE IV

*Effect of washed platelets as an "accelerator" of the activation of AcG-poor prothrombin*

Two and five-tenths ml. vol. "thrombic mixtures" (T) containing borate buffer (pH = 7.75), 0.1 ml. Pro. (0.1%), 0.25 ml. $CaCl_2$ (M/20), and activators noted. Clotting times (sec.) of 0.5 ml. $F_2$ + 0.25 ml. T (at stated incubation periods): 26°C

| | Activators *ml.* (dilution) | Incubation time (*min.*) | | | | |
|---|---|---|---|---|---|---|
| | | 5′ | 10′ | 20′ | 40′ | 60′ |
| 1. | Tpln., 0.25 | 420″ | 300″ | 202″ | 141″ | 110″ |
| 2. | Tpln., 0.25<br>Plat., 0.1 | 390″ | 240″ | 80″ | 22.4″ | 15″ |
| 3. | Tpln., 0.25<br>AcG, 0.1 (0.001%) | 300″ | 140″ | 52″ | 25.8″ | 20.3″ |
| 4. | Tpln., 0.25<br>AcG, 0.1 (0.1%) | 11.4″ | 9.4″ | 8.0″ | 7.8″ | 7.6″ |

Table IV is a typical experiment to illustrate how little "AcG-like" action was found in these well-washed platelet preparations. The poor thrombin formation in tests 1 indicates the deficiency of AcG in the chosen prothrombin, so that optimal calcium and tissue thromboplastin are insufficient activators in the hour incubation period. Compare the excellent activation in 4, with plenty of AcG added. Platelets (tests 2) produced relatively little effect, roughly comparable to the weaker AcG addition in 3, which amounts to a concentration of only 1:2,500,000 in the final thrombic mixture.

Tested on thrombin-fibrinogen mixtures, our platelets showed, if anything, a few seconds' prolongation of clotting times and no "fibrinoplastic" enhancement of clotting at several dilutions. We did find some evidence that a commonly encountered instability of thrombin solutions was apt to be less in the presence of platelets, however.

### *Effects of* Varying *Fibrinolysin Concentrations (Platelets Constant) and of* Varying *Platelet Concentrations (Fibrinolysin Constant) on Activation of Purified Prothrombin*

These experiments were performed by our usual technic of following the activation of prothrombin by means of the clotting times for fibrinogen ($F_2$) of samples of the various thrombic mixtures, removed after stated incubation periods. The 2.5 ml. mixtures contained 0.1 ml. Pro. (0.1%), 0.25 ml. $CaCl_2$ (50mM), 0.1 ml. AcG (0.1%), and the activators studied. From the series of data obtained, only the clotting-time values *at a single incubation period* were selected for comparing the effects of varying amounts of the fibrinolysin or platelet activators, and the accompanying graphs are plotted in this way.

In Figure 1 the three curves A, B, C are, respectively, the clotting times (sec.) of samples tested after one, five, and 60 min. incubation. The mixtures all contained 0.1 ml. platelet preparation but the fibrinolysin concentration was varied and is expressed as mg. per 2.5 ml. of the mixture. Curve $B^1$ is from another experiment and, therefore, not quantitatively comparable with B, but, by extending the lysin concentration up to 9 mg. it brings out the additional point of an "optimum" for the enzyme effect at about 2.5 mg. (per 2.5 ml.) in this particular experiment. The data clearly show that the "potentiating" effect of fibrinolysin is more marked on the earlier phases of prothrombin activation and does depend upon the enzyme concentration, but, according to the 60 min. data of Figure 1, C, does not significantly influence the final thrombin yield (shortest clotting times).

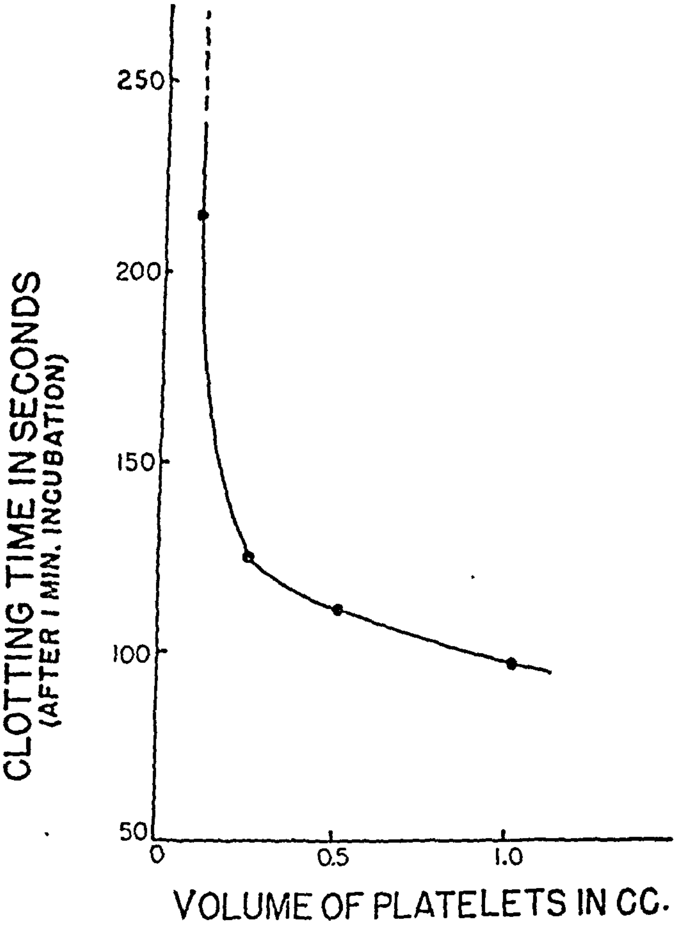

FIG. 2. EFFECT OF VARYING PLATELET CONCENTRATION WITH FIBRINOLYSIN CONSTANT

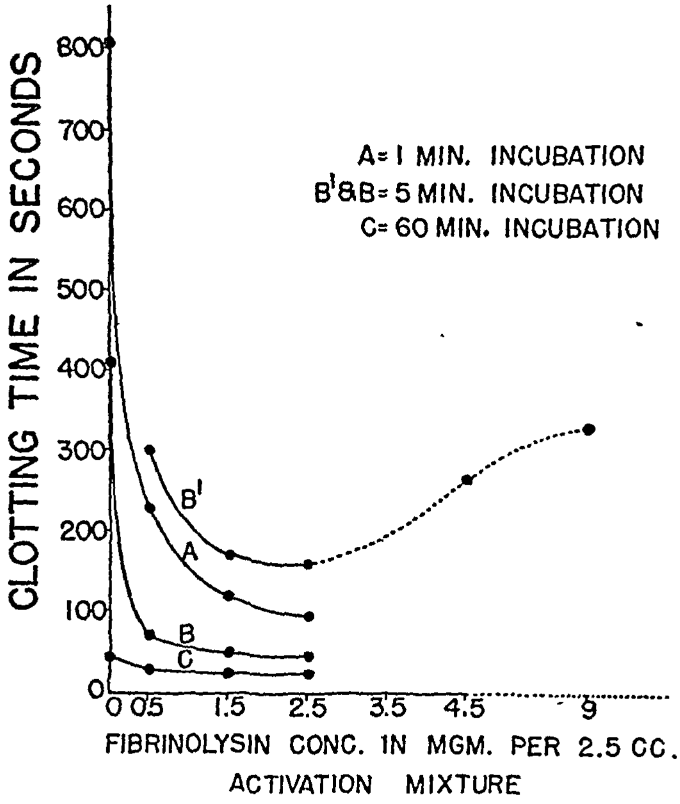

FIG. 1. EFFECT OF VARYING FIBRINOLYSIN CONCENTRATION WITH PLATELETS CONSTANT

*Figure 2 is an experiment with fixed and approximately optimal fibrinolysin (2.5 mg./2.5 ml.) but varying the amount of platelet preparation between 0 and 1.0 ml./2.5 ml. of thrombic mixture. The one min. incubation samples are selected for the clotting-time test data plotted.*

Figure 3 shows controls (five min. and 60 min. incubation samples) in an experiment with varying platelets (volumes plotted logarithmically) and no fibrinolysin present. The clotting times are significantly longer than when lysin is added and are expressed in minutes.

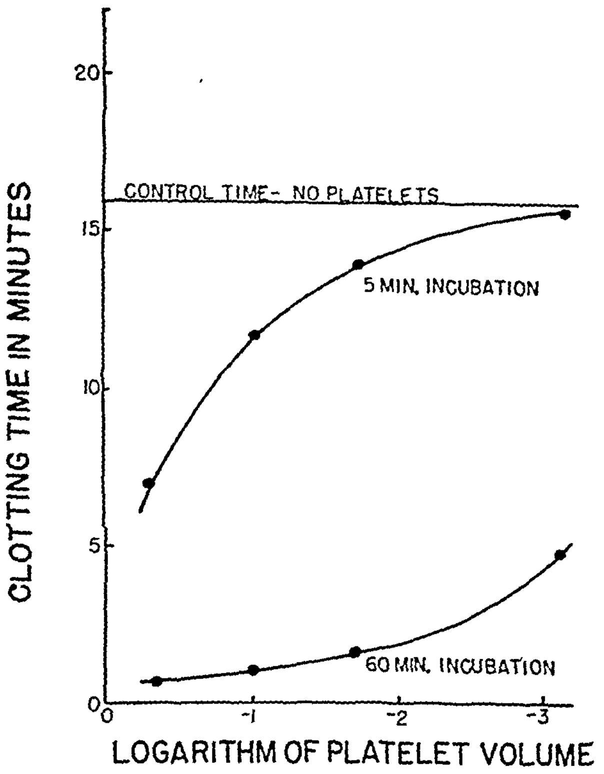

FIG. 3. EFFECT OF VARYING PLATELET CONCENTRATION; NO FIBRINOLYSIN PRESENT

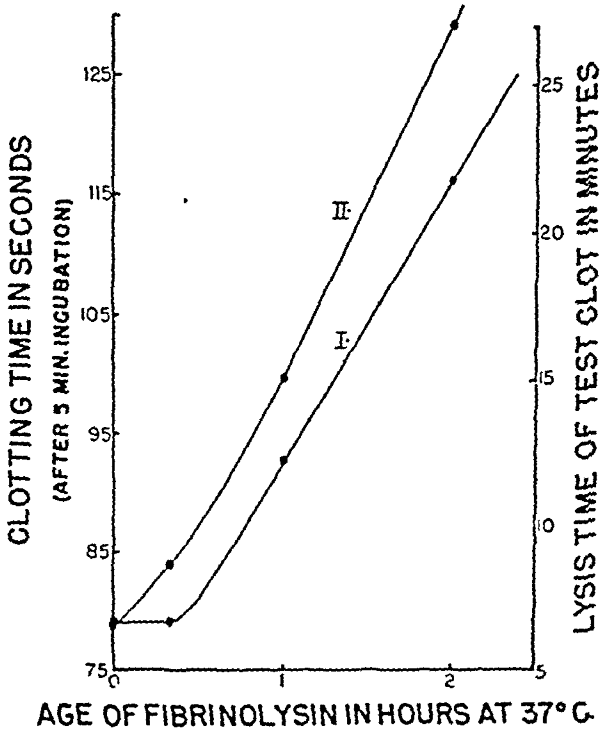

FIG. 4 LOSS OF THROMBOPLASTIC (I) AND LYTIC (II) ACTIVITY OF PROTEASE

### *Effects of Thermal Instability of Fibrinolysin on its Lytic Potency and on its Platelet Thromboplastin Potentiating Effects*

Loomis' fibrinolysin (1% solution in borate buffer) was allowed to undergo deterioration at 37°C (water bath) and samples. removed at stated intervals of aging, tested simultaneously for *1*) fibrinolytic potency, by the method of Lewis and Ferguson (13), and *2*) platelet thromboplastin potentiating effect, in tests closely similar to those described above. Figure 4 plots, against age of lysin (up to two hours at 37°C), *I.* fibrinolysis times, which lengthen with weakening enzyme potency, and *II.* clotting times of five min. incubation samples, which, according to data of the preceding section, give a relative measure of "thromboplastic" potency. It would appear that the two activities of the enzyme preparation weaken at very similar rates. If the clotting-time data are plotted against residual fibrinolytic activity (*units* computed from a reference curve [13]), the figure will resemble the descending portion of curves B and B¹ of Figure 1, in which dilution, instead of deterioration, was the method of reducing the enzyme activity.

### *Effects of Protease-Inhibitors on Conversion of Prothrombin to Thrombin, in Presence of Brain Thromboplastin, Platelets, and Fibrinolysin*

Preliminary to these studies, extensive experiments were carried out clearly to establish that *1*) none of the antienzymes inhibited the thrombin-fibrinogen reaction, *2*) fibrinolysin (0.1%) had little, if any, effect on strong thrombin ($>$ 1 u./ml.) but could lengthen clotting times with weak thrombins ($<$ 0.1 u./ml.) due to the opportunity afforded for *fibrinogen*olysis (*cf.* [4]).

Proceeding to the testing of thrombin formation from purified prothrombin with added AcG and optimal calcium and other reagents stated, the data of Table V record a typical series of experimental results. Interpreting the clotting-time tests in the usual way, the results indicate most significantly that pancreatic inhibitor (PI) and serum antifibrinolysin (AFin) are *not* inhibitory to the cited thromboplastic agents (tpln. A or plat.-lysin mixtures) even though they prevented clot lysis in the fibrinolysin-containing tub-

TABLE V

*Effects of antiproteases on thromboplastic agents*

One-tenth ml. Pro. (0.1%), 0.1 ml. AcG (0.1%), 0.25 ml. Ca (M/20), activators and inhibitors stated, and borate buffer to 2.5 ml. vol. Clotting times (sec.) of 0.25 ml. mixture + 0.5 ml $F_1$, at stated incubation times, 25°C.

| | Activators *ml.* (conc.) | Inhibitors *ml.* (conc.) | Incubation time (*min.*) | | | | |
|---|---|---|---|---|---|---|---|
| | | | 5′ | 10′ | 20′ | 40′ | 60′ |
| 1. | Tpln. A, 0.25 | 0 | 22.5″ | 17.1″ | 15.2″ | 15.0″ | 14.2″ |
| 2. | Tpln. A, 0.25 | SBI, 0.1 (0.01%) | 450″ | 420″ | 450″ | 450″ | 380″ |
| 3. | Plat., 0.1<br>Lysin, 0.2 (0.5%) | 0 | 49″ | 25.2″ | 21.8″ | 21.3″ | 21″ |
| 4. | Plat., 0.1<br>Lysin, 0.2 (0.5%) | SBI, 0.1 (0.1%) | 630″ | 135″ | 42″ | 38.5″ | 35.4″ |
| 5. | Tpln. A, 0.25 | 0 | 25.5″ | 21.1″ | 19.2″ | 18.4″ | 19.6″ |
| 6. | Tpln. A, 0.25 | PI, 0.1 (0.1%) | 28.8″ | 23.3″ | 21.8″ | 21.5″ | 20″ |
| 7. | Plat., 0.1<br>Lysin, 0.15 (1%) | 0 | 29.1″ | 23″ | 22″ | 22″ | 22″ |
| 8. | Plat., 0.1<br>Lysin, 0.15 (1%) | PI, 0.1 (0.1%) | 28″ | 22.5″ | 19.9″ | 20″ | 20″ |
| 9. | Tpln. A, 0.25 | 0 | 22.5″ | 15.4″ | 14.6″ | 13.1″ | 12.2″ |
| 10. | Tpln. A, 0.25 | *AFln, 0.15 (0.5%) | 28.5″ | 22.4″ | 20.6″ | 21.2″ | 21″ |
| 11. | Plat., 0.1<br>Lysin, 0.15 (1%) | 0 | 58.2″ | 29.3″ | 24.6″ | 24.3″ | 25″ |
| 12. | Plat., 0.1<br>Lysin, 0.15 (1%) | *AFln, 0.15 (0.5%) | 37.6″ | 24.9″ | 22.4″ | 22.2″ | 20″ |

* A 15 min. incubation period, at 26°C, of antifibrinolysin with thromboplastic agent, was used to permit development of its antienzyme effect (13).

whereas inhibitor-free controls all lysed within 12–24 hours. Soybean inhibitor (SBI) was exceptional in inhibiting the brain thromboplastin (tests 2), so that it evidently has some "antithromboplastic" action, despite which its effect on activation with plat.-lysin (tests 4) is strongly inhibitory only in the earlier activation phases.

### *Effects of Antiproteases on Thromboplastic Action of Trypsin-Platelet Mixtures*

Similar experiments to those in the preceding section were performed with pancreatic trypsin (tryp.) instead of fibrinolysin, and with the antiproteases PI and AFln previously used. The results are given in Table VI. Here platelets alone (tests 1) and trypsin alone (tests 2) gave very slow and inadequate activation of the prothrombin-AcG-Ca mixture, whereas plat. + tryp. (tests 3) exerted a marked thromboplastic effect on the thrombin formation. In contrast to the previous results with fibrinolysin, the trypsin synergism with platelets was apparently completely blocked by both the tested inhibitors, PI (tests 4) and AFln (tests 5).

TABLE VI

*Effects of antiproteases on thromboplastic action of trypsin-platelet mixtures*

Two-tenths ml. Pro. (0.1%), 0.2 ml. AcG (0.1%), 0.5 ml. Ca (M/20), activators and inhibitors stated, and borate buffer to 5 ml. vol. Clotting times (sec.) for 0.25 mixture + 0.5 $F_1$ at stated incubation periods (25°C).

| | Activators *ml.* (conc.) | Inhibitors *ml.* (conc.) | Incubation time (*min.*) | | | | |
|---|---|---|---|---|---|---|---|
| | | | 5′ | 10′ | 20′ | 40′ | 60′ |
| 1. | Plat., 0.1 | 0 | 720″ | 540″ | 270″ | 100″ | 65″ |
| 2. | Tryp., 0.3 (0.01%) | 0 | 182″ | 140″ | 95″ | 58″ | 51″ |
| 3. | Plat., 0.1<br>Tryp., 0.3 (0.01%) | 0 | 47″ | 29.5″ | 26.5″ | 23.5″ | 23.2″ |
| 4. | Plat., 0.1<br>Tryp., 0.3 (0.01%) | PI, 0.1 (0.1%) | 660″ | 240″ | 175″ | 113″ | 96″ |
| 5. | Plat., 0.1<br>Tryp., 0.3 (0.01%) | AFln, 0.3 (4%) | 710″ | 490″ | 205″ | 103″ | 71″ |

### *"Thromboplastic" Tests with Enzymes* (sans *Platelets*) *and Anti-Enzymes*

Numerous tests on prothrombin, AcG, calcium mixtures, with fibrinolysin and with trypsin, but without any platelet or other added thromboplastin, merely confirmed the all but negligible "thromboplastic" effects of the enzymes alone. None of the inhibitors (SBI, PI, AFln), acting alone, have significant effects except for some inhibition of the very minor thrombin formation (seen in controls) by SBI. The differences with enzyme + inhibitor combinations were slight and chiefly a matter of failure to inhibit the minor actions of fibrinolysin with PI and AFln, and the blocking of minor actions of 0.01% trypsin. There is no need to cite these experimental data in detail.

### *Is There Thrombin in the Fibrinolysin Preparation?*

It is extremely difficult to prepare fibrinolysin without significant contamination with thrombin. Loomis' serum enzyme preparation was selected for the present studies particularly because it appeared to fulfill the claim (16) to be free from thrombin and prothrombin. Owing to fibrinogenolysis, however, it is difficult to put this to an exacting test, but we sought to overcome this difficulty by neutralizing 0.3 ml. 1% lysin with 0.3 ml. 0.5% antilysin (Loomis') and testing on recalcified prothrombin-free fibrinogen ($F_2$). Even this test was inconclusive, however, as a small clot did appear in about two hours, persisting for at least four days (at 26°C). The antilysin alone caused no clot.

TABLE VII

*Effects of weak thrombin on platelet thromboplastin*

One-tenth ml. Pro. (0.1%), 0.1 ml. AcG (0.1%), 0.25 ml. Ca (M/20), activators cited, with borate buffer to 2.5 ml. vol. Clotting times (sec.) for 0.25 mixture + 0.5 ml. $F_1$, at stated incubation times (26°C).

| | Activators (ml.) | Incubation time (min.) | | | | |
|---|---|---|---|---|---|---|
| | | 5′ | 10′ | 20′ | 40′ | 60′ |
| 0. | 0 | 885″ | 913″ | 920″ | 970″ | 1010″ |
| 1. | Plat. (0.1) | 810″ | 620″ | 330″ | 190″ | 97″ |
| 2. | Plat. (0.1)<br>Thr. (0.25) | 145″ | 52″ | 22″ | 12″ | 9″ |
| 3. | Plat. (0.1)<br>0.5% lysin (0.3) | 60″ | 46″ | 15″ | 12″ | 9″ |

At the end of this time it had formed a potent thrombin, the activity of which was not increased by incubation with thromboplastin, indicating that unaltered prothrombin was no longer present. Several dilutions of the lysin- and thromboplastin-free product were examined for the platelet thromboplastin potentiating action. In tests 2 of Table VII an effect, qualitatively and approximately quantitatively similar to that obtained with 0.3 mg. fibrinolysin (tests 3), is noted. When freshly prepared, this particular thrombin dilution clotted the test fibrinogen in 750 seconds, but its potency decreased fairly rapidly on standing. Owing to this thrombin instability, exact control tests, *minus* the platelets, were impracticable. Such tests were attempted, however, and did show, in a reasonably satisfactory manner, that the thrombin itself caused only insignificant shortening of the control (tests 1) clotting times.

AcG, Ca system, with platelets, all proved negative. The fractions were then shaken with chloroform, following the old method (17, 18) for activating fibrinolysin.

*Tests on $CHCl_3$-treated dialysates:* the usual testing for potentiation of platelet thromboplastin was again carried out, with the result that the only significant acceleration of thrombin conversion was found in Fraction C (30–40% $[NH_4]_2SO_4$).

*Control tests:* I. $CHCl_3$-treated Fraction C, in same dilution as in preceding tests, showed (*a*) traces of thrombin, *e.g.*, 0.25 ml. C(dil.) + 0.5 ml. $F_2$: clotting time (26°C) 575 sec.; (*b*) some fibrinolytic action, *e.g.*, lysis of foregoing clot in about 12 hours. II. The other $CHCl_3$-treated fractions showed no evidence of thrombin or lysin.

In view of the data of the preceding section (Table VII), the trace of thrombin in $CHCl_3$-treated Fraction C, as well as the fibrinolysin, and possibly other possible factors remaining to be detected, all need critical evaluation.

It was surprising to find any thrombin at all in the above material. Since $CHCl_3$ is known to inactivate antithrombin, we performed some tests which showed a considerable reactivation of thrombin which had lost practically all its activity after incubating 24 hours at room temperature with (*a*) Seitz-filtered plasma (S.Pl.) or with (*b*) $BaSO_4$-adsorbed serum. A thrombin-destroying action of $CHCl_3$, seen also with thrombin solution alone, partly (at least) explains the *partial* recovery. It was found, incidentally, that pancreatic inhibitor retards the antithrombic action and permits the subsequent $CHCl_3$ treatment to give bettter thrombin recoveries.

## DISCUSSION

The present investigations confirm earlier observations that both trypsin and serum fibrinolysin (plasmin or tryptase) exert significant clot-aiding effects attributable to participation in the mechanisms of conversion of prothrombin to active thrombin. With purified prothrombin, optimal calcium, and such accessory factors as are supplied with added "serum accelerator globulin," but no added thromboplastin (cell- or tissue-factor), these enzyme preparations have little, if any, "thromboplastic" action of their own, the clotting times of the thrombic mixtures plus purified fibrinogen solution being too long to give significant results. In previous studies (4) with potent tissue thromboplastins, clotting times in control tests, without enzyme, were too short to give more than suggestive evidence that addition of these proteases could "potentiate" the thromboplastic action, particularly in its earlier phases. In the present studies, well-washed platelet preparations, shown to be without significant effects other than those of a relatively weak "thromboplastic" character, were markedly potentiated by the enzyme materials in question.

This PTP ("platelet thromboplastin potentiating") effect of the serum fibrinolysin parallels its lytic activity both in dilution experiments and during deterioration at 37°C. However, protease inhibitors from pancreas (PI) and from serum (AFln) do not abolish the PTP effect, although they do inhibit the enzymic fibrinolysis.

Soybean inhibitor (SBI) has additional "antithromboplastic" effects, even for tissue- and platelet-thromboplastins without proteolytic enzyme (Table V), so that its effect of at least partly inhibiting the PTP action may very well be due to its antithromboplastic instead of its antilytic effects.

In the case of trypsin, however, all the inhibitors abolish the two effects simultaneously. Thus, the arguments we have previously (4) built up for a "thromboplastic enzyme" mechanism accessory to the thrombin-forming phase of blood clotting, are now still valid only in the artificial test systems with pancreatic trypsin. Failure of correlation of the two effects in tests with appropriate inhibitors (serum antifibrinolysin especially) overthrows a large amount of evidence otherwise strongly suggesting a similar role for the natural serum protease fibrinolysin. The conclusion must be that some other factor must be sought for in the enzyme preparation to account for its thromboplastin potentiating effects.

The possibility that this may be a trace of thrombin is perhaps suggested by the discovery (Table VII) of a significant "PTP" effect of very small amounts of thrombin such as may frequently be found as contaminants of serum enzyme and many other blood preparations. However, the extremely minute and possibly doubtful traces of thrombin demonstrable in some special tests with the present lysin, would appear insufficient to invoke this explanation here. Nor is it probable that the minute traces of thrombin, present or appearing in the prothrombin-AcG mixtures (which are very dilute), can be implicated, particularly to explain the sharp *increase* in "thromboplastic" action when the enzyme-platelet activator is used and compared with control tests. It is barely possible, however, that these sources of thrombin trace-contaminant may require some consideration in explaining (fully) the thromboplastic action of platelets alone (*i.e.,* without enzyme) as seen in the data of Tables III and IV.

Ware, Fahey, and Seegers (14) found some thrombin formation in platelet-activated mixtures containing stronger prothrombin (330 u./ml.) but none in an hour's incubation with much lower (1.34 u./ml.) prothrombin. If their prothrombin contained the usual trace of thrombin contaminant, these results at the higher concentrations would appear similar to ours, whereas at the lower concentrations the thrombin contaminant may very well have been diluted to insignificance.

Our preliminary attempts to prepare a "platelet thromboplastin potentiating" factor, from apparently thrombin- and prothrombin-free Seitz filtered (× 5) plasma, succeeded only in the case of a $CHCl_3$-treated 30–40% saturated $(NH_4)_2SO_4$ Fraction C, in which definite traces of both fibrinolysin and thrombin were demonstrated. The surprising finding of some thrombin here raises some new practical questions. Our suggested explanation is that some thrombin formation in artificially treated plasmas may not be detected, yet result in a "metathrombin" (combination of thrombin with natural antithrombin) from which thrombic activity is recoverable under certain circumstances, *e.g.,* following treatment with chloroform. Many questions as to the state of both prothrombin and thrombin in plasma and in serum are opened up by these new facts and some special studies on these matters are being pursued and have already been mentioned in a preliminary communication (19).

On casual observation, our PTP factor might appear to be acting like the so-called "accelerator" agents, two and possibly more types of which have occupied the attention of a number of recent investigators A recent paper (20) in this JOURNAL adequately reviews the current status of these factors and particularly gives experimental evidence that Seegers' "serum accelerator globulin" preparation contains *both* Alexander's SPCA ("serum prothrombin conversion accelerator") *and* 'labile factor" As we have mentioned earlier, the routine addition of a reasonable amount of serum AcG in our test systems should, we believe, ensure a sufficiency of these "accelerator" factors and make it unnecessary to enter into any discussion of them in the present connection. Rather, we may proceed to consideration of our results in terms of the 'thromboplastic" problem

This does bring the present work into relation with some other studies in the coagulation literature, particularly those which give attention to the idea of a thromboplastin precursor or "prothrombokinase" (Collingwood and MacMahon, 1912; Widenbauer, 1943) requiring activation in order to provide the "thromboplastic" (thrombokinase) factor for the clotting mechanism. In a series of recent papers Milstone reviews this literature and presents his new experimental data. His first paper appeared while our present studies were nearing completion and offered evidence of the enhancement of prothrombin conversion to thrombin in the presence of platelets when a (crude) plasma "globulin" preparation was added. In discussion Milstone (21) states: "Conceivably, platelets might contain prothrombokinase as suggested by Collingwood and MacMahon, or a platelet enzyme might activate the prothrombokinase ('thromboplastinogen') of plasma as suggested by Quick (1947). The present experiments do not decide between these hypotheses, nor do they eliminate other possibilities." Milstone's second paper (22) contributes some new technics for analyzing the mechanisms of blood coagulation into three phases. A "prothrombokinase" preparation from a euglobulin precipitate of bovine plasma served for the study of *plasma* thrombokinase (thromboplastin) formation, with the aid of calcium. A noteworthy point in discussion is . . . "thrombin does not catalyze the activation of prothrombin; but its possible effect on the activation of prothrombokinase remains undetermined." Milstone (23) prepared from the $BaSO_4$ adsorbate of bovine plasma globulins a material ("converter fraction") which . . . "hastened both the activation of prothrombin and the conversion of crude prothrombokinase are obviously many points of confirmation and parallel interest between Milstone's work and ours, both the present and previously published (4). However, these serve only to emphasize the complexities of the problem and the final solution must await much future investigation.

## CONCLUSIONS

1. Crytalline trypsin and "purified" serum fibrinolysin accelerate clotting of ordinary recalcified plasma and platelet-free plasma obtained with the silicone technic.

2. These enzymes accelerate and increase thrombin yields from heat-defibrinated plasma or other crude prothrombins.

3. They have no significant effects on the thrombin-fibrinogen reaction (apart from lytic interference) nor upon the activation of purified prothrombin with Ca and AcG but negligible thromboplastin.

4. They markedly potentiate platelet thromboplastic action.

5. *Well washed* platelets are thromboplastic, but negligibly AcG-like, and nonfibrinoplastic.

6. The platelet thromboplastin potentiating (PTP) effect of fibrinolysin shows an optimal enzyme concentration and lessens roughly parallel to weakening of lytic potency by dilution or by thermal deterioration.

7. It is, however, not abolished by effective antiproteases (antilytic) from pancreas (PI) and serum (antifibrinolysin), and only incompletely by soybean inhibitor (SBI), which, however, is antithromboplastic.

8. The PTP effects of trypsin, on the other hand, are completely abolished by the antilytic agents.

9. Very small amounts of thrombin (down to a limit) also have a PTP action, but the fibrinolysin used was practically thrombin-free.

10. A crude plasma protein fraction obtained at 30–40% $(NH_4)_2SO_4$ saturation of "prothrombin-free" (Seitz-filtered, × 5) plasma, when treated with $CHCl_3$, had PTP effects but contained some thrombin as well as fibrinolysin.

11. While many of these data support Ferguson's theory (1) of a clot-aiding role of "thromboplastic enzymes," perhaps still valid for trypsin, the inhibitor data controvert this for fibrinolysin. After considering the possible significance of thrombin, there remains the possibility of a new PTP factor, possibly the "prothrombokinase converter factor" postulated by Milstone (*op. cit.*), but apparently unrelated to the "accelerator" factors about which much work has appeared in the recent coagulation literature.

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# *"...the only drug we have seen that allays anxiety without clouding consciousness"*

J.A.M.A. 140:672 (June 25) 1949

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TO 53(5)

# EFFECT OF DIABETES AND INSULIN ON THE MAXIMUM CAPACITY OF THE RENAL TUBULES TO REABSORB GLUCOSE[1]

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(Submitted for publication September 5, 1950; accepted, October 30, 1950)

Glycosuria occurs whenever the amount of glucose filtered by the glomeruli exceeds the reabsorptive capacity of the tubules. The glycosuria of diabetes, therefore, is dependent not only on the degree of hyperglycemia but also on the concurrent glucose reabsorptive capacity.

A number of observers have noted that diabetic patients sometimes may have little or no glycosuria in spite of hyperglycemia (1, 2). Mirsky and Nelson (2) have attributed this phenomenon to decreased glomerular filtration rate, especially among elderly patients, or to an increase in the glucose reabsorptive capacity of the tubules.

Glucose reabsorption depends on an active transport system which is undoubtedly enzymatic in nature (3) and, therefore, is limited by a maximum rate, designated $Tm_G$. This transport system appears to be subject to a variety of functional controls including hormones. Indeed, slight reduction in $Tm_G$ after the administration of insulin has been observed in the dog (4), while thyroid extract has been noted to increase $Tm_G$ (5).

The present study represents an evaluation in man of the effects of diabetes and of insulin on the maximum ability of the tubules to reabsorb glucose.

### SUBJECTS AND METHODS

Glomerular filtration rate (GFR) and $Tm_G$ were studied in 12 diabetic patients. Nine non-diabetic subjects served as controls. Pertinent clinical data are summarized in Table I. The duration of diabetes was not known with certainty in each instance, but was mild to moderate

TABLE I

*Clinical data*

| Patient | Sex | Age | Surface area | Known duration diabetes | Insulin requirements | Fasting plasma glucose | Blood pressure | Urine | Fundi |
|---|---|---|---|---|---|---|---|---|---|
| | | | *(sq. m.)* | *(years)* | *(units per day)* | *(mgm. per cent)* | *(mm. Hg)* | *(protein)* | |
| 1 | M | 49 | 1.62 | 1 | 10 PZI | 120 | 110/74 | 0 | normal |
| 2 | M | 61 | 1.91 | 2 | none | 123 | 112/68 | 0 | normal |
| 3 | M | 37 | 1.75 | 7 | 35 PZI | 204 | 120/90 | 5 mm. per cent | normal |
| 4 | M | 38 | 1.83 | 3 | 40 PZI, 15 Reg. | 287 | 128/70 | 0 | slight sclerosis vessel |
| 5 | M | 53 | 1.71 | 2 | none* | 368 | 160/80 | 0 | normal |
| 6 | M | 43 | 1.91 | 23 | 80 Reg. | 455 | 120/80 | 0 | normal |
| 7 | F | 45 | 1.70 | 3 | 5 Reg. | 115 | 130/80 | 0 | normal |
| 8 | F | 73 | 1.74 | 1 | none | 145 | 160/90 | 0 | sclerosis vessels |
| 9 | F | 65 | 1.64 | 9 | 15 PZI | 159 | 140/90 | 0 | sclerosis vessels |
| 10 | F | 45 | 1.64 | 7 | 10 PZI | 173 | 145/80 | 1 + | few patches exudate |
| 11 | F | 43 | 1.62 | 3 | 5 Reg. | 200 | 120/88 | 5 mm.% | slight A-V nicking |
| 12 | F | 40 | 1.68 | 16 | 45 PZI, 20 Reg. | 480 | 120/80 | 0 | normal |
| 13 | M | 43 | 1.65 | Non-diabetic | | | | normal | |
| 14 | M | 27 | 1.87 | Non-diabetic | | | | normal | |
| 15 | M | 53 | 1.72 | Non-diabetic | | | | normal | |
| 16 | M | 48 | 1.95 | Non-diabetic | | | | normal | |
| 17 | M | 23 | 1.66 | Non-diabetic | | | | normal | |
| 18 | M | 47 | 1.74 | Non-diabetic | | | | normal | |
| 19 | F | 44 | 1.78 | Non-diabetic | | | | normal | |
| 20 | F | 28 | 1.52 | Non-diabetic | | | | normal | |
| 21 | F | 25 | 1.67 | Non-diabetic | | | | normal | |

* Resistant to subcutaneous insulin, treated by diet alone.

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[1] This work was supported by a grant from the U. S. Public Health Service.

in severity as evidenced by the daily insulin requirements and the fasting plasma glucose levels. None of the diabetic patients had established hypertensive disease. Minimal proteinuria was observed in two patients, while one patient had slight proteinuria and a few patches of exudate in the fundi. Mild to moderate sclerosis of the retinal vessels was observed in four patients.

All experiments were performed in the post-absorptive state. Insulin therapy was witheld for 24 hours prior to a study. In each study $Tm_G$ was measured for three or more consecutive periods. In 15 of the 21 studies, inulin clearance was measured for three 15 to 20 minute periods prior to the start of the glucose infusions. Priming doses of 50 per cent glucose in water were given intravenously in amounts calculated to raise the plasma glucose to between 500 and 800 mgm. per cent. The plasma glucose level was maintained by a constant intravenous infusion delivered by a calibrated pump. The sustaining infusion for the diabetic patients contained 15 to 25 per cent glucose and was delivered at 3 ml. per minute. Twenty minutes were allowed for equilibration between the start of sustaining infusion and the beginning of the ten minute urine collections.

In the insulin experiments, regular Lilly or Squibb preparations were used. Twenty units were given intravenously as a priming dose while 2 units per minute were delivered in the sustaining infusion. Again, 20 minutes were allowed for equilibration before the start of the ten minute collection periods.

Inulin clearance was used as a measure of glomerular filtration rate. The plasma inulin level was maintained between 30 and 40 mgm. per cent. All urines were collected through a multi-eyed catheter. The bladder was washed out at the end of each period except when the urine flow exceeded 10 ml. per minute. Arterial bloods were obtained at the mid point of each period.

Inulin was determined by the Harrison modification (6) of the Alving, Rubin and Miller method (7). Prior to the inulin measurement, glucose was removed from each 1 ml. sample of plasma and diluted urine by exposure for 30 minutes to 6 ml. of a 30 per cent yeast suspension. Glucose was determined by a modification (4) of the Folin method. Glycolysis was inhibited by the addition of a small amount of fluoride to each blood sample, which was centrifuged immediately. The plasma was drawn off within 15 minutes of collection.

TABLE II

*Plasma glucose levels and ratios of glucose load to $Tm_G$*

All plasma glucose levels done on arterial blood. Initial and final plasma glucose levels are recorded for the three periods that made up each observation, except those marked* which consisted of five periods and those marked† which consisted of four ten minute periods.

| Patient | Plasma glucose, *mgm. per cent* | | Ratio glucose load to $Tm_G$ | |
|---|---|---|---|---|
| | $Tm_G$ alone | $Tm_G$ + Insulin | $Tm_G$ alone | $Tm_G$ + Insulin |
| 1 | 615-735 | 703 675 | 2.01 | 2.48 |
| 2 | 450 420 | 420-402 | 1.31 | 1.33 |
| 3 | 551 616 | 616 516 | 1.54 | 1.69 |
| 4 | 567 606 | 548 533 | 1.75 | 1.93 |
| 5 | 470 480 | 460 440 | 1.25 | 1.24 |
| 6 | 690 721 | 683 613 | 2.12 | 1.92 |
| 7 | 605-610 | 610 550 | 1.71 | 1.91 |
| 8 | 438-425 | 425-318 | 1.43 | 1.58 |
| 9 | 624-636* | 610-550* | 1.77 | 1.76 |
| 10 | 624-654* | 666-545* | 1.62 | 1.65 |
| 11 | 765-840* | 840-780* | 2.30 | 2.59 |
| 12 | 885-900 | 930-870 | 2.55 | 2.06 |
| | | Average | 1.64 | 1.70 |
| 13 | 910 990 | — | 2.56 | — |
| 14 | 590-600 | 620 510 | 2.52 | 2.15 |
| 15 | 1022-1035* | 1401-1368* | 3.44 | 4.38 |
| 16 | 585-600† | 585-585† | 1.77 | 2.02 |
| 17 | 504-500 | — | 1.78 | — |
| 18 | 812-798† | 770-693 | 2.98 | 2.68 |
| 19 | 580-630 | — | 1.72 | — |
| 20 | 410-460 | — | 1.44 | — |
| 21 | 660-717 | — | 2.34 | — |
| | | Average | 2.28 | |

## *Plasma Glucose Levels and the Ratios of Glucose Load to $Tm_G$*

The ranges of arterial plasma glucose levels achieved during the measurement of $Tm_G$ alone and of $Tm_G$ during the administration of insulin are recorded in Table II. Generally the plasma glucose level was between 500 and 800 mgm. per cent and rarely varied more than 50 mgm. per cent during the measurement of $Tm_G$ alone. Following the administration of insulin the plasma glucose level with one exception declined in spite of the continued glucose infusion. The fall in plasma glucose during the measurement of $Tm_G$ while insulin was being administered averaged 57 mgm. per cent with a range of 0 to 121 mgm. per cent. A glucose load to $Tm_G$ ratio of 1.5 or higher is generally accepted (4) as being adequate to completely saturate the normal tubular reabsorptive capacity for glucose. Ratios exceeding this value were achieved in all but two of the diabetic patients and in all but one of the non-diabetic subjects. The lowest ratio of 1.25 occurred in a patient with an unusually high $Tm_G$. Any error introduced by a too low load of glucose would result in a falsely low $Tm_G$. Because of the reduction in $Tm_G$ resulting from insulin administration, the glucose load to $Tm_G$ ratio was maintained after insulin, even though the load decreased because of the falling plasma glucose level.

## RESULTS

The values for glomerular filtration rate (GFR), $Tm_G$ and the ratio of GFR to $Tm_G$ observed in 12 diabetic and in nine normal subjects are recorded in Table III along with normal values established by Smith (8). GFR and $Tm_G$ values are corrected to a surface area of 1.73 sq. m.

The group of normal subjects is not large, but their filtration rates and $Tm_G$s are in the normal-range established by Smith and his associates (8), with the exception of one $Tm_G$ (patient 20, Table III) which exceeded the mean normal plus 2 standard deviations ($375 + 2 \times 79.7 = 543$ mgm. per minute for males, $303 + 2 \times 55.3 = 414$ mgm. per minute for females).

The diabetic group is also small and, although the mean $Tm_G$ for both males and females are greater than the corresponding normal values, the differences are not statistically significant. However, $Tm_G$ exceeded the mean normal plus 2 standard deviations in five of the 12 diabetic patients. Application of the binomial theorem indicates that the possibility of this occurring by chance alone is remote. Although patients with hypertension or those who had frank renal disease were excluded from the study, two of the female patients with low filtration rates (patients 8 and 12) were the only diabetics to have $Tm_G$s less than the mean normal value.[2]

TABLE III

*Glomerular filtration rate, $Tm_G$ and ratio of filtration rate to $Tm_G$ in non-diabetic and diabetic subjects*

Glomerular filtration rate (GFR) and $Tm_G$ values are corrected to a surface area of 1.73 sq. m.

| Group | Patient | Glomerular filtration rate | $Tm_G$ | GFR $Tm_G$ |
|---|---|---|---|---|
| | | *(ml. per min.)* | *(mgm. per min.)* | |
| Normal Male | Smith (8) | 131. ±21.5 | 375. ±79.7 | 0.371. ±0.0563 |
| | 13 | 132 | 521 | 0.25 |
| | 14 | 133 | 306 | 0.44 |
| | 15 | 125 | 372 | 0.34 |
| | 16 | 148 | 470 | 0.32 |
| | 17 | 125 | 354 | 0.35 |
| | 18 | 146 | 402 | 0.36 |
| | Average | 135 | 404 | 0.34 |
| Normal Female | Smith (8) | 117. ±15.6 | 303. ±55.3 | 0.395 ±0.0617 |
| | 19 | 117 | 414 | 0.28 |
| | 20 | 143 | 422 | 0.34 |
| | 21 | 111 | 322 | 0.34 |
| | | — | — | — |
| Diabetic Male | 1 | 120 | 415 | 0.29 |
| | 2 | 143 | 514 | 0.28 |
| | 3 | 104 | 402 | 0.26 |
| | 4 | 112 | 377 | 0.30 |
| | 5 | 164 | 619 | 0.26 |
| | 6 | 138 | 450 | 0.31 |
| | Average | 130 | 463 | 0.28 |
| Diabetic Female | 7 | 120 | 438 | 0.27 |
| | 8 | 91 | 271 | 0.34 |
| | 9 | 117 | 419 | 0.28 |
| | 10 | 118 | 461 | 0.26 |
| | 11 | 140 | 495 | 0.28 |
| | 12 | 65 | 226 | 0.29 |
| | Average | 103 | 386 | 0.29 |

TABLE IV

*Effect of insulin on $Tm_G$ and the ratio of glomerular filtration rate (GFR) to $Tm_G$*

| Group | Patient | $Tm_G$, *mgm. per min.* | | | GFR/$Tm_G$ | | |
|---|---|---|---|---|---|---|---|
| | | Control | Insulin | Δ | Control | Insulin | Δ |
| Diabetic | 1 | 415 | 352 | −63 | 0.29 | 0.37 | +0.08 |
| | 2 | 514 | 496 | −18 | 0.28 | 0.32 | +0.04 |
| | 3 | 402 | 361 | −41 | 0.26 | 0.29 | +0.03 |
| | 4 | 377 | 314 | −63 | 0.30 | 0.36 | +0.06 |
| | 5 | 619 | 539 | −80 | 0.26 | 0.28 | +0.02 |
| | 6 | 450 | 427 | −23 | 0.31 | 0.29 | −0.02 |
| | 7 | 438 | 394 | −44 | 0.27 | 0.32 | +0.05 |
| | 8 | 271 | 197 | −74 | 0.34 | 0.41 | +0.07 |
| | 9 | 419 | 401 | −18 | 0.28 | 0.30 | +0.02 |
| | 10 | 461 | 420 | −41 | 0.26 | 0.27 | +0.01 |
| | 11 | 495 | 445 | −50 | 0.28 | 0.31 | +0.03 |
| | 12 | 226 | 204 | −22 | 0.29 | 0.22 | −0.27 |
| | Average | 424 | 379 | −45 | 0.27 | 0.31 | +0.04 |
| Non-Diabetic | 13 | 330 | 216 | −114 | 0.44 | 0.57 | +0.13 |
| | 14 | 372 | 396 | +24 | 0.34 | 0.32 | −0.02 |
| | 15 | 470 | 416 | −54 | 0.32 | 0.35 | +0.03 |
| | 16 | 402 | 404 | +2 | 0.36 | 0.36 | −0.00 |

Normally glomerular and tubular functions are closely correlated and the ratio of glomerular filtration rate to $Tm_G$ is perhaps the most desirable means for comparison of $Tm_G$ among different groups of subjects. The average GFR/$Tm_G$ ratios of the normal male and female subjects shown in Table III do not differ significantly from Smith's normal series. The ratios for the diabetic patients, however, are low. When the diabetics are compared to Smith's normal group, the t values for the critical ratios are 5 for the males and 4 for females. These values are indicative of a highly significant increase of $Tm_G$ in relation to the glomerular filtration rate among the diabetics as compared to normals.

The difference between the male diabetic group and the normal group recorded in Table IV is barely significant, and the difference between the female diabetics and the three normal females is not significant. However, the normal groups in Table IV are so small that the comparison with Smith's larger series of normal subjects appears to be the more valid. The measurements of this study were made by techniques identical with those of Smith (8).

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[2] Three diabetic patients with frank renal disease were also studied but excluded from this report. These patients had glomerular filtration rates of 39, 45, and 70 ml. per

rate was measured before the start of the glucose infusions necessary for the Tm$_G$ observations. The filtration rate increased 1 ml. per minute on the average in the non-diabetic group, and decreased 7 ml. per minute in the diabetic group.

The administration of insulin reduced the Tm$_G$ acutely in all 12 diabetic subjects studied (Table IV). The decrease ranged from 18 to 20 mgm. per minute with an average of 45 mgm. per minute. This represented an 11 per cent decrease. The ratio of GFR/Tm$_G$ increased in 10 of the 12 subjects. This was mainly the result of changes in Tm$_G$ although slight increases in filtration rate contributed to the effect in several patients. The latter decreased only 1 ml. per minute on the average during the insulin periods as compared to those obtained during the measurement of Tm$_G$ without insulin. A progressive fall in filtration rate with no obvious explanation was noted throughout the study in one of the exceptions (patient 12). Insulin was also administered to four non-diabetic subjects (Table IV). A decrease in Tm$_G$ occurred in two of these subjects.

Tm$_G$ was measured in one diabetic patient during seven consecutive periods without the administration of insulin. The total length of this study was equal to the longest study in which insulin was administered. Throughout this control study the GFR/Tm$_G$ ratio was constant and the difference between the lowest and the greatest Tm$_G$ values was 26 mgm. per minute.

## DISCUSSION

Five of 12 patients with mild to moderately severe diabetes, uncomplicated by known renal disease, were found to have Tm$_G$ values greater than the accepted normal range.[3] Tm$_G$ was above the mean normal in 10 of the patients. The two exceptions had decreased glomerular filtration rates although hypertension, proteinuria or other evidences of renal disease were absent. The reduction in renal functions in these patients may have been due to arteriosclerosis of the kidneys, or perhaps represented the early stages of intercapillary glomerulosclerosis.

The ratio of filtration rate to Tm$_G$ was significantly reduced among the diabetic patients. This appeared to be the result of increased Tm$_G$ values rather than of decreased glomerular filtration rates, for in the majority of patients the filtration rates were in the normal range. In addition, the low filtration rate to Tm$_G$ ratios were not due to possible alterations in glomerular activity induced by the glucose infusions.

The administration of insulin decreased the Tm$_G$ in each of 12 diabetics tested and increased the GFR/Tm$_G$ ratio toward normal in 10 of the 12. Insulin decreased the Tm$_G$ in two of four non-diabetic subjects. Although slight decreases in plasma glucose levels usually occurred after insulin administration, this was kept at a minimum by the sustaining infusion of glucose. In any case, adequate glucose load to Tm ratios were maintained throughout the studies.

The decreased Tm$_G$ after insulin was not due to increased utilization of glucose by the renal tubules for this would be reflected by an *apparent increase* in Tm$_G$. Whether the effect of insulin on the Tm$_G$ was due to insulin itself or to a contaminant such as the "hyperglycemic factor" (10) is not certain. However, in the present experiments and in the doses used, insulin given intravenously did not raise the blood glucose values.

An increase of Tm$_G$ in diabetes might be attributed to insulin deficiency itself or might be secondary to other hormonal imbalances that may play a role in diabetes. The reduction of Tm$_G$ effected by insulin is not readily explicable on the basis of the usual concepts of insulin action. Offhand, a greater ability of the renal tubule cells to transport glucose would be expected. But in any case, the decreased Tm$_G$ after insulin may explain clinical observations in diabetics that are reported from time to time such as: "after prolonged insulin therapy the urine may contain sugar even though the blood sugar is much lower than before treatment and the urine may have been negative before insulin therapy was instituted" (1).

Since insulin decreases Tm$_G$ in diabetics, it is possible that the differences between the GFR/Tm$_G$ ratios of diabetics and non-diabetics may be the result of increased secretion of endogenous insulin in the non-diabetic occasioned by the rather large loads of glucose. This endogenous insulin would lower the $Tm_G$ perhaps maximally and additional exogenous insulin would thus have little or no effect. In line with this hypothesis was the failure of insulin to reduce $Tm_G$ in two of the four normal subjects tested. In the diabetics the glucose load does not call forth as much insulin, and exogenous insulin would be free to exert its ability to reduce $Tm_G$. In view of the equilibration period allowed to achieve relatively stable plasma glucose levels, this point could not be critically examined. However, a downward trend in $Tm_G$ was not apparent in successive periods among the non-diabetic subjects.

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[3] Richardson (9), working independently, has found the Tm$_G$ to be greater than the normal range in six of 14 diabetic patients.

## CONCLUSIONS

1. The maximum capacity of the renal tubules to reabsorb glucose is above the normal range in a significant proportion of patients with mild to moderately severe diabetes.
2. When related to the glomerular filtration rate, the glucose reabsorptive capacity is increased in diabetics without renal disease.
3. Insulin reduces the glucose reabsorptive capacity of diabetics.

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# THE HAEMOLYTIC FACTOR IN THE ANAEMIA OF LYMPHATIC LEUKAEMIA[1]

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(Submitted for publication June 24, 1950; accepted, November 6, 1950)

Cases of lymphatic leukaemia complicated by a haemolytic anaemia have been reported by Haden (1), Singer and Dameshek (2), Wiseman (3), Feldman and Yarvis (4) and by Stats, Rosenthal and Wasserman (5) among others. It has been the commonly held view that such cases are rare and that the cause of the anaemia usually found in association with lymphatic leukaemia lies in a disturbance of erythropoiesis the result of replacement of erythropoietic tissue by leukaemic tissue. That haemorrhage is also an etiological factor in the anaemia in some cases is clear to everyone, but that excessive haemolysis may also be a factor is by most authors considered an improbability. Because of the haemosiderosis which they found and the hyperplasia of erythropoietic tissue which was demonstrated in some of their cases, earlier workers (6–8) believed that excessive haemolysis probably played an important role. This view was disputed by Forkner (9) who cited the work of Whipple and Robscheit-Robbins (10) which showed that there was only a little more iron in leukaemic than in normal livers. Recently the problem has been reviewed by Collins and Rose (11) who admit that excessive haemolysis may be present but state that in the case of lymphatic leukaemia the anaemia is due in most cases to depression of erythropoiesis.

It has been the purpose of the present investigation to determine by means of the differential transfusions of Ashby whether or not excessive haemolysis is an important factor in the production of the anaemia of lymphatic leukaemia even when the usual signs of excessive haemolysis are absent and whether in any cases there is evidence of the presence of a haemolytic process not seen in normal subjects.

## EXPERIMENTAL METHOD

The cases studied were transfused with 500–1,500 cc. of stored whole blood. The blood had been stored for one to four days in the proportion of 400 cc. of blood to 150 cc. of an anticoagulant mixture containing 2.3 gm. dextrose U.S.P. and 1.7 gm. sodium citrate U.S.P. per 100 cc. The inagglutinable erythrocytes were counted the day after transfusion, then every other day for 10 days and subsequently at intervals of 10 to 14 days. The method of differential agglutination was that described by Dacie and Mollison (12). With this method it has been found that the standard error of the difference between duplicate observations is of the order of 6% of the inagglutinable count (13). In the present study four counts were made on each specimen, and in some cases two counts by two observers. The results have been expressed numerically in the manner described by Brown and associates (13) who worked on the hypothesis that in some cases of anaemia two processes of erythrocyte destruction could be distinguished and who named these the *linear haemolytic mechanism* and the *exponential haemolytic mechanism*. This method of description provides *1*) an estimate of the average life of the transfused erythrocytes ($\bar{t}$), *2*) the fraction of the transfused erythrocytes which has been destroyed by the *exponential haemolytic mechanism* ($F_e$) and *3*) a fictitious average life which would have obtained if the linear haemolytic mechanism had been acting alone ($\bar{t}_s$). These are derived from the equation.

$$\frac{N}{N_0} = (1 - L_s t)\,\frac{R \exp(-L_e t) + 1}{R + 1}\text{[4]}$$

which has been found to be the simplest equation which reasonably fits the data in those cases where the plotted results show considerable curvature. When the decay curve is linear the equation becomes

$$N = N_0(1 - L_s t)$$

It has been found by Callendar and colleagues (14) that in a normal recipient the decay curve is linear and that the average life of the transfused erythrocytes is about 60 days. This corresponds well with the estimates of life span of the erythrocyte which have been made by those who fed a normal subject glycine containing $N^{15}$ (15) and by those who have used pigment excretion studies as the basis for estimation (16).

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[1] This work was supported by grants-in-aid from the Medical Division of the National Research Council.

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[3] Research Fellows

[4] Notation

$N$ = Donor cell count in recipient at time t, million per c.mm.

$N_0$ = Donor cell count in recipient immediately after transfusion, million per c.mm.

$t$ = Time measured from transfusion (days)

$\left.\begin{array}{l}L_s\\ L_e\\ R\end{array}\right\}$ = Constants.

$\bar{t}$ = Average life of transfused cells measured from $t = 0$ (days)

$\bar{t}_s$ = Average life corresponding to linear component (days)

The cases which are reported now were not selected except with respect to suitability of blood group and accessibility during the period of observation.

## RESULTS

Case I, J. H. (48-3506), a man 67 years of age had been a known diabetic for 10 years. For one year before admission he had complained of dyspnoea and substernal distress on exertion and latterly of nocturnal dyspnoea. Examination on admission showed moderate enlargement of the the cervical lymph nodes, enlargement of the liver, a heart which was borderline in size, rales over the lower half of both lung fields, and fluid in the right pleural cavity. Urinalysis—trace of albumen, varying glycosuria. HGb. 5.8 gm.%. R.B.C. 1,740,000. Haematocrit 18%. W.B.C. 650 with 58% lymphocytes, 16% prolymphocytes, 4% lymphoblasts, 20% polymorphonuclear neutrophiles and 2% band cells. Reticulocytes 2.4%. Platelets 31,680. Serum Bilirubin 0.4 mg.%. Fasting Blood Sugar 231 mg.%. Blood Urea 33 mg.%. Serum Chlorides 586 mg.%. Serum Proteins 6.3 gm.%. with 4.12 gm. albumin and 2.18 gm. globulin. Plasma Prothrombin Content 62%. Faecal Urobilinogen 631 mg./day during a three-day collection by the method of Schwartz, Sborov and Watson (17). Bone marrow smear showed 6% small lymphocytes, 25.5% large lymphocytes, 14.5% prolymphocytes, 42% lymphoblasts and an L/E ratio of 9/1. Chest x-ray showed a pneumonic infiltration of both lung roots and increased markings in the upper half of the left lung. C/T ratio of 16.5/30. Electrocardiogram—chronic myocardial pathology. He was treated with rest, digitalis, insulin, a low

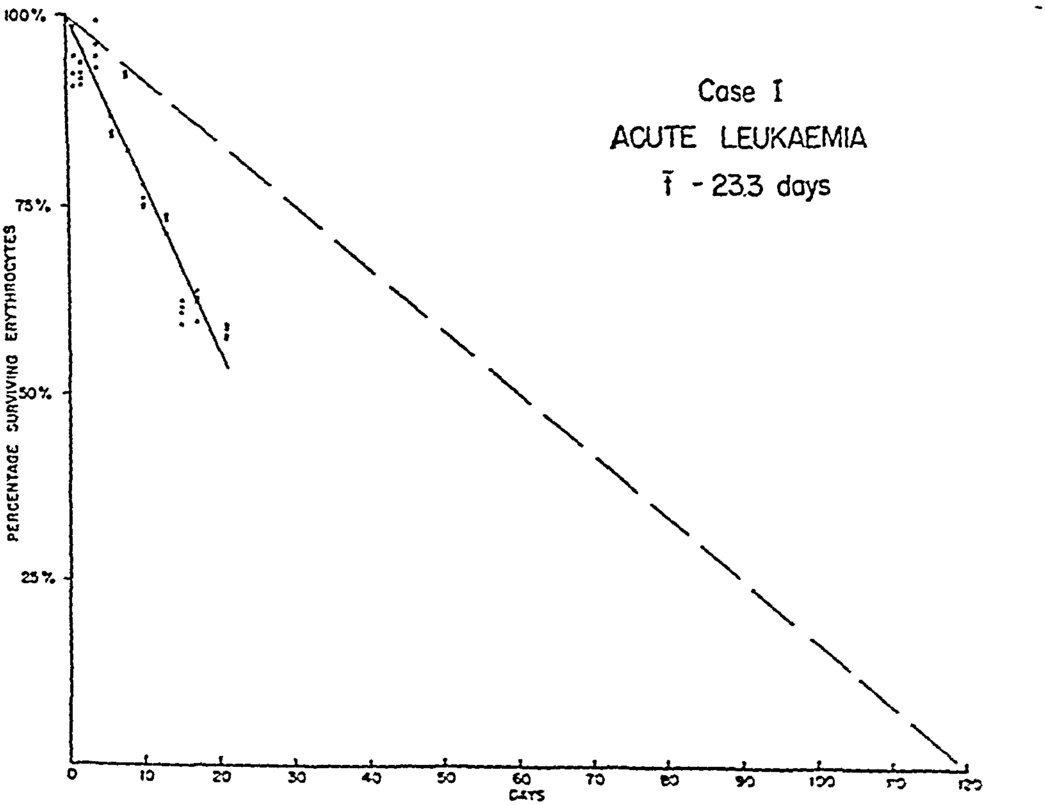

FIG. 1. SURVIVAL OF TRANSFUSED ERYTHROCYTES IN CASE I

The dots represent the actual observations and the solid line has been drawn through points calculated from the fitted quotation. The broken line represents the decay curve in a normal subject.

sodium neutral ash diet, and Fowler's solution. The diabetes mellitus was brought under good control. He died at home eight weeks after his admission to hospital.

The test transfusion was followed for 21 days. When the number of surviving erythrocytes was plotted against time the decay curve was found to be linear for that period (Figure 1). It showed an increased slope as compared with that found in normal subjects (14) and the calculated average life of the transfused cells ($\bar{t}$) was 23.3 days.

Case II, W. H. (27913), a man aged 54 had been suffering from disseminated sclerosis for 13 years. For six years he had been aware of swellings in his axillae and groins and for one year there had been a mass in his abdomen. He also complained of pallor, increasing dyspnoea on exertion and of oedema of the ankles. Examination showed a pale wasted man with generalized lymphadenopathy, a palpable liver and a greatly enlarged spleen. There was the characteristic evidence of moderately advanced disseminated sclerosis but for two years there had been no increase in the disability from this cause. Urinalysis was negative. HGb. 4.4 gm.%. R.B.C. 1,850,000. Haematocrit 17%. W.B.C. 1,050,000 with 94% lymphocytes. Reticulocytes 1.0%. Platelets 60,000. Serum Bilirubin less than 0.2 mg.%. He lived for about 18 months following this admission to hospital.

The test transfusion was followed for 38 days and the plotted results showed considerable curvature (Figure 2). The calculated average life of the transfused cells ($\bar{t}$) was 18 days and the proportion destroyed by the *exponential haemolytic mechanism* ($F_e$) was 0.34.

Case III, C. W. (49–4189), a man aged 68 had noted loss of strength and lumps in his neck for one year. There were enlarged lymph nodes to be palpated in the cervical, occipital, axillary, epitrochlear, inguinal and popliteal regions. The liver edge was just palpable and the spleen was enlarged to below the level of the umbilicus. Urinalysis negative. HGb. 9.1 gm.%. R.B.C. 3,200,000

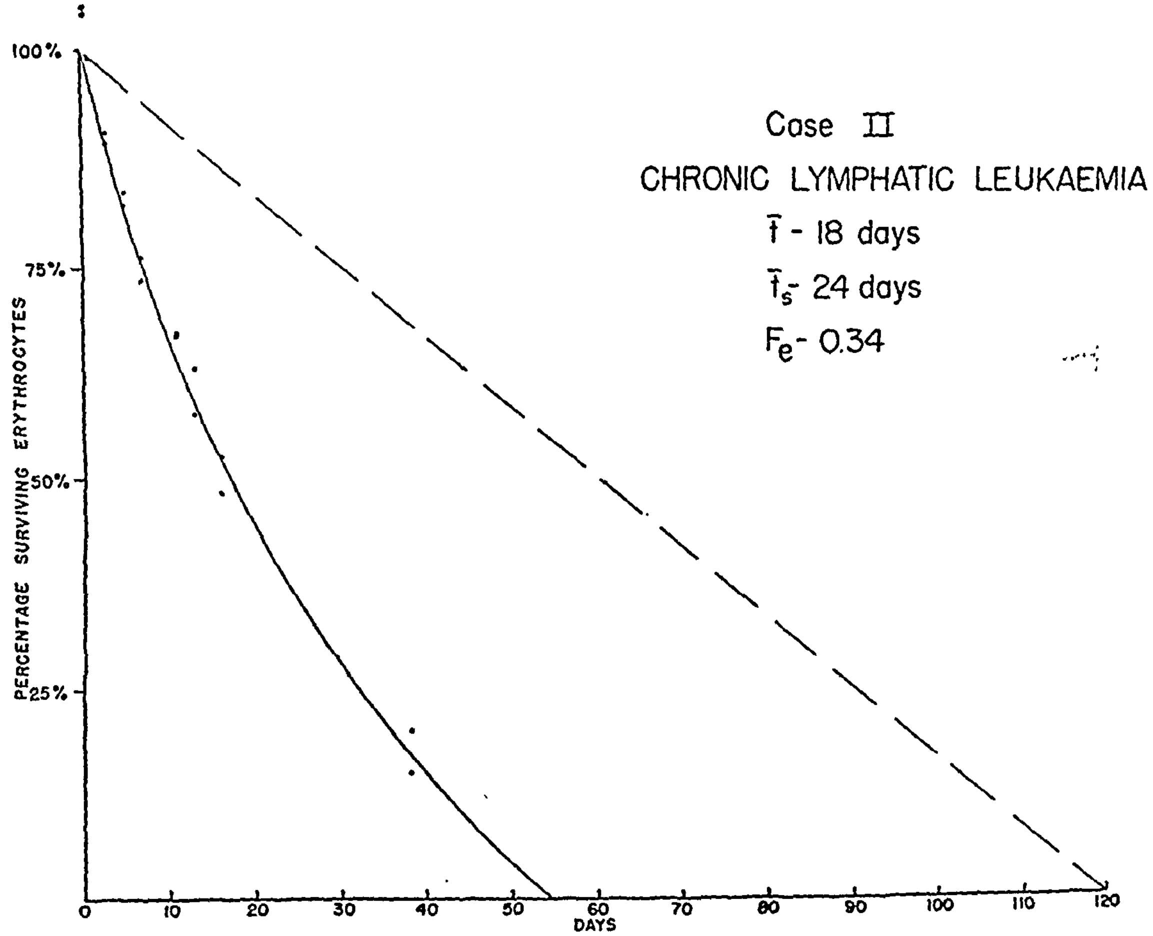

FIG. 2. SURVIVAL OF TRANSFUSED ERYTHROCYTES IN CASE II

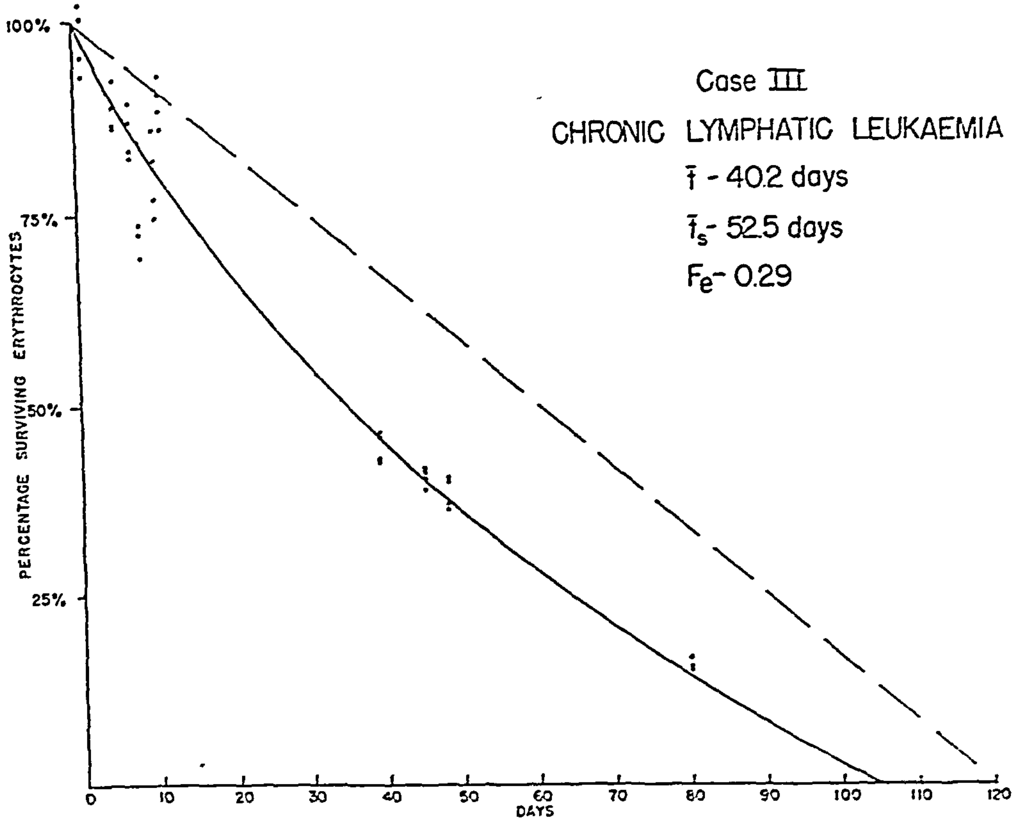

FIG. 3. SURVIVAL OF TRANSFUSED ERYTHROCYTES IN CASE III

Haematocrit 29%. W.B.C. 29,800 with 96% lymphocytes, 2% polymorphonuclear neutrophiles, 1% band cells, 1% myelocytes. Reticulocytes 1.1%. Platelets 169,000. Serum Bilirubin 0.4 mg.%. Faecal urobilinogen 31.9 mg./day. Lymph node biopsy showed the characteristic picture of lymphatic leukaemia. He was given deep x-ray therapy amounting to 300r over both supraclavicular and both axillary regions during a period four to eight days following his test transfusion. Radiotherapy was then discontinued because his white blood count had fallen to 10,000. He received further radiotherapy six weeks later. He is still alive, a year after the test transfusion.

The plotted results in this case showed an increased slope and also some curvature (Figure 3). The fraction of the transfused cells which

TABLE I

*Blood counts following the subcutaneous injection of 1 cc. 1/1,000 adrenalin in Case IV*

| | Before | 5 minutes | 10 minutes | 20 minutes | 35 minutes |
|---|---|---|---|---|---|
| HGb., gm. % | 10.4 | 10.4 | 10.6 | 10.7 | 10.6 |
| R.B.C. | 3,320,000 | 3,230,000 | 3,420,000 | 3,480,000 | 3,250,000 |
| W.B.C. | 187,000 | 355,000 | 299,000 | 298,000 | 342,000 |
| Lymphocytes, % | 97 | | 81 | 75 | 77 |
| Smudge cells, % | | | 18 | 21 | 15 |
| Lymphoblasts, % | | | | 1 | 1 |
| Monocytes, % | 1 | | | | |
| Polymorphs. % | 2 | | 3 | 3 | 7 |
| Platelets | 172,600 | 329,400 | 1,555,000 | 174,000 | 435,500 |

had been destroyed by the *exponential haemolytic mechanism* ($F_e$) was estimated to be 0.29 and the average life of the transfused cells ($\bar{t}$) was 40.2 days.

Case IV, F. E. B. (49–9730), a man aged 68 gave a history of fatigue and weakness for three years and increasing dyspnoea on exertion for one year. For a month he had had a productive cough. He was pale and emaciated and showed a generalized lymphadenopathy and moderate enlargement of the spleen. There was marked arteriosclerosis of the Monckeberg type and his heart was grossly enlarged. There were numerous coarse rales to be heard over the lower lobes of both lungs. Urinalysis negative. HGb. 6.95 gm.%. R.B.C. 2,340,000. Haematocrit 22%. W.B.C. 330,000 with 97% lymphocytes, 2% polymorphonuclear neutrophiles, 1% eosinophiles. Reticulocytes 1.4%. Platelets 730,080. Blood counts following the injection of 1 cc. 1/1000 adrenalin are shown in Table I. Serum Bilirubin 0.5 mg.%. Faecal Urobilinogen 56.8 mg./day. Serum N.P.N. 48 mg.%. Plasma Protein 6.49 gm.% with 3.96 gm. albumin and 2.53 gm. globulin. Sputum contained large numbers of lymphocytes, some of them lying in sheets. Chest x-rays showed persistent diffuse linear streaking and some mottling in the lower halves of both lung fields. Two sternal punctures yielded no marrow. Treatment with urethane was begun nine days after his test transfusion and in the next three weeks there was considerable improvement with a gain in weight, an increase in haemoglobin from the post-transfusion level of 11 gm.% to 12.1 gm.% and a fall in white blood count to 182,000. The improvement continued with a daily dose of 4.5 gm. urethane so that after three months his white blood count had fallen to 23,000. His haemoglobin at that time was 11.1 gm.%. His lung fields were clear on x-ray.

The result of the transfusion in this case was entirely within normal limits. The decay curve was strictly linear and interrupted the base line at 114 days.

## DISCUSSION

To say that evidence of an increased rate of destruction of transfused erythrocytes is evidence of an increased destruction of the patient's own cells is to assume that the two are treated by the body in the same way. That this is not always the case has been shown in congenital haemolytic icterus (18), nocturnal haemoglobinuria (12) and sickle-cell anaemia (19). In these conditions transfused cells from a normal donor may survive for a normal length of time, though it is obvious that the patient's own cells are being destroyed abnormally rapidly. In these three conditions, the reverse also holds, and the patient's cells when transfused into a normal recipient are destroyed more rapidly than the cells of a normal donor. Save in these conditions there is no evidence that cells from a normal donor are destroyed in the recipient faster than the recipient's own cells provided there are not present in the recipient antibodies against the donor cells (mis-matched transfusion) and also, possibly, provided the total erythrocyte count is not raised by transfusion above the recipient's normal count. Such evidence would be admittedly difficult to obtain, and the point of identical treatment of the donor's and the recipient's cells, with the exceptions already mentioned, cannot be proven. It can be said, however, that in those cases in this and in previous work (13, 20) where there was the conventional evidence of excessive haemolysis, *viz.* reticulocytosis, and increase in serum bilirubin and faecal urobilinogen, the average life of the transfused cells has always been markedly diminished. It can also be pointed out that the inferences to be drawn from the present work involving the transfusion of cells from normal donors into abnormal recipients, followed on a comparison of the results of work involving the transfusion of cells from normal donors into normal recipients (14).

The suggestion has been made that in patients with enlarged spleens, some of the transfused erythrocytes may be "lost" in the spleen giving a falsely low survival time, but there seems no reason to suppose that the transfused erythrocytes are more likely to be contained in the spleen than are the patient's own cells. That both may disappear from the peripheral circulation into the spleen is obvious. If the transfused erythrocytes were indiscriminately "lost" in the splenic pulp, an increase in the exponential component of the decay curve would follow and there would be no increase in the activity of the *linear haemolytic mechanism* if indiscriminate "loss" in the spleen were the only abnormal process at work. Data which would obtain in this situation have not been found in this or earlier series (13, 20). Abnormally rapid removal of the transfused erythrocytes by the spleen from the main mass of circulating erythrocytes according to some characteristic of the erythrocytes themselves, such as age, would result in a linear decay curve with increased slope. Any significant return of these cells to the systemic circulation would cause upward deviations of the decay curve which have not yet been observed. In summary rebuttal of the suggestion that an enlarged spleen may vitiate the results in the present experiments, it may be said that there is no reason to suppose the transfused erythrocytes are removed from the circulation more rapidly by the spleen than are the patients' own cells and that if both are removed abnormally rapidly there is no evidence to date that they are returned to the circulation in significant numbers.

In three of the four cases of lymphatic leukaemia which have been studied, the transfused erythrocytes were destroyed at a rate faster than normal, which is interpreted as evidence of excessive haemolysis in these cases. What part, if any, the urethane therapy had in the results of Case IV, it is impossible to say. During the period of observation his haemoglobin was maintained at the immediate post-transfusion level and there was a marked improvement in his general condition, with diminution in size of his spleen and accessible lymph nodes. He was, in fact, in a remission. In Cases II and III the reticulocyte count and the serum bilirubin, and in Case III the faecal urobilinogen, were within normal limits, despite the definite increase in rate of destruction of transfused erythrocytes. This suggestion that the present experimental method is a more sensitive indicator of excessive haemolysis than those more commonly employed has been presented before (20). In Cases II and III there was also evidence of activity of the *exponential haemolytic mechanism*. The *exponential haemolytic mechanism* has not been seen in normal recipients except when the post-transfusion erythrocyte count has been above normal and it has not been evident in this or previous series except when there has also been increased activity of the *linear haemolytic mechanism*. In Case I, the acute case, where the observed portion of the decay curve was sensibly linear, it should be remembered that the period of observation was only 21 days and that further counts might have provided evidence of the *exponential haemolytic mechanism*.

TABLE II

*Survival of transfused erythrocytes in four cases of lymphatic leukaemia*

| Case No. | Average life ($\bar{t}$ days) | Average life corresponding to linear mechanism ($\hat{t}_s$ days) | Fraction destroyed by exponential mechanism ($F_e$) |
|---|---|---|---|
| I | 23.3 | 23.3 | 0 |
| II | 18 | 24 | 0.34 |
| III | 40.2 | 52.5 | 0.29 |
| IV | 57 | 57 | 0 |

These results would seem to show that in at least some cases of lymphatic leukaemia the anaemia is contributed to by increased haemolysis and that in some cases there is evidence not only of increased haemolysis but also of haemolysis of an abnormal type. It is not necessary in three of the present four cases to explain the anaemia entirely on the basis of diminished erythropoietic activity, and indeed in Case II where the rate of haemolysis was more than three times that of normal, the haemolytic activity was of an order which might entirely explain the anaemia found. The fact that excessive haemolysis has been demonstrated in cases where the more usual indices of haemolysis have been lacking suggests that it may be an important factor more often than is now believed. *In what proportion of cases this holds,* remains to be determined by further work. The physiological mechanism and the sites of the different types of haemolysis which have been observed also remain speculative. The question of a relation between the two types of haemolytic activity which have been demonstrated here and the activity of immune bodies which have been demonstrated in some cases of haemolytic anaemia in leukaemia (21) will have to be answered by further work.

## ACKNOWLEDGMENT

Acknowledgement is made of the technical assistance of Mrs. Shirley Davey and Mr. George Cragg.

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# THE PRECIPITABLE IODINE OF SERUM IN NORMAL PREGNANCY AND ITS RELATION TO ABORTIONS[1]

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(Submitted for publication August 2, 1950; accepted, November 6, 1950)

In 1948 it was reported by Heinemann, Johnson and Man (1) that early in pregnancy the precipitable (protein-bound) iodine in the serum (SPI) rose to concentrations in the upper range of normal or even to levels that outside of pregnancy are characteristic of hyperthyroidism. It also appeared that if the SPI did not rise, the pregnancy was likely to end in abortion within the first four months. Some evidence was presented that such accidents might be prevented by the administration of active thyroid substance. The present paper deals with a further investigation of the phenomenon.

## MATERIAL

Patients from the Clinics of the Department of Obstetrics and Gynecology in the New Haven Hospital form part of the material studied. In addition, analyses of the serum of patients have been made for members of the Clinical Faculty who have permitted the data to be used and have generously contributed the clinical information required for the purposes of this investigation. Finally, a certain number of patients with disorders of function of the thyroid were drawn from the Metabolism Clinic.

In many cases only single observations are available. In some, determinations of SPI were made at intervals throughout pregnancy. In a limited number observations were continued into the early postpartum period. For obvious reasons, figures preceding pregnancy were only occasionally obtained. In a certain proportion of cases serum lipids were also measured.

## METHODS

Venous blood was drawn, with precautions against contamination by iodine, from patients in the postabsorptive state or after a meal free from fat (fruit juice, black coffee, and toast, either dry or with jam). Serum was removed under anaerobic precautions. Lipids were measured by the method of Man and her associates (2–6). Until July, 1949, SPI was measured by the method of Riggs and Man (7); after that date by a micromodification (8). Analytical results by the two methods agree.

## *RESULTS*

*Normal pregnancy.* In Figure 1 are presented the values for SPI obtained on one or more occasions from 46 normal women during the course of pregnancies that proceeded to term without complications. None of these patients received thyroid or any medication known to affect thyroid function during their pregnancies or in the periods immediately preceding or following pregnancy. Four had goiters without evidence of abnormal activity of the thyroid. Of the 56 determinations from the end of 16 weeks until term, SPI was only twice lower than 5.6 $\gamma$ per cent, and in all but three instances was 6.0 $\gamma$ per cent or higher. Values lower than 6.0 $\gamma$ per cent were only transitory incidents. The average concentration of SPI in nonpregnant normal persons is 5.0 $\gamma$ per cent (8). In the last 24 weeks of normal pregnancy, therefore, SPI was invariably above the average normal value for nonpregnant persons. It is not uncommon to find values up to 10 $\gamma$ per cent which, outside of pregnancy, would denote severe hyperthyroidism. In pregnancy, however, such concentrations are associated with no symptoms, signs, nor metabolic disorders indicative of overactivity of the thyroid gland.

In the same figure is illustrated the course of SPI in the progress of pregnancy in a series of patients in whom repeated observations were made. There is no definite tendency for SPI to rise or fall during the last 24 weeks. It is, however, subject to fluctuations far greater than those that have been observed in nonpregnant individuals. Variations of 2 $\gamma$ per cent over relatively short intervals are frequent and variations of as much as 4 $\gamma$ per cent have been encountered, with-

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[1] This investigation was supported (in part) by a research grant from the U. S. Public Health Service.

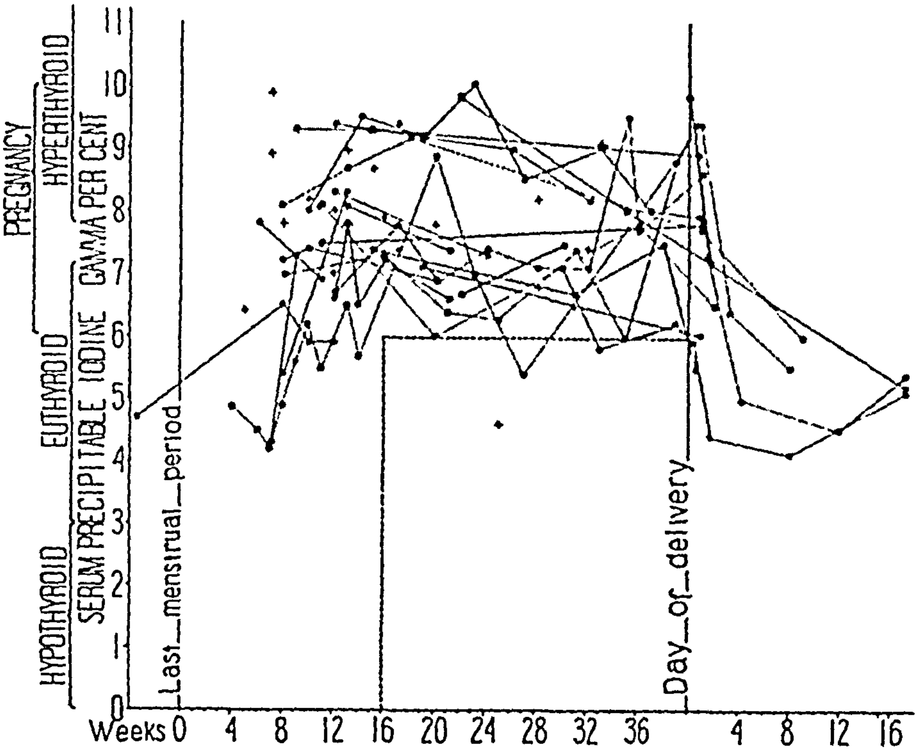

FIG. 1. SERUM PRECIPITABLE IODINE (SPI) IN THE COURSE OF NORMAL PREGNANCY

Crosses represent single observations. Circles connected by lines represent repeated observations.

out any discernible disturbances. In normal individuals SPI is quite stable (8, 9).

In many or most instances the SPI rises sharply at or near the onset of pregnancy. Concentrations in the hyperthyroid range have been obtained as early as six weeks after the last menstrual period. In only one case was an observation obtained preceding pregnancy. In this case SPI had risen from 4.7 to 6.5 $\gamma$ per cent by the time the first measurement in pregnancy was taken, eight weeks after the last mentrual period. In a certain proportion of subjects, however, the rise is distinctly delayed; and in most it continues after the initial sharp increase. The same large fluctuations observed during the later part of pregnancy are evident in these early stages. Sometimes, as a result of these fluctuations, SPI, after the initial rise, drops down again temporarily into the nonpregnant range.

After delivery SPI diminished again, returning to normal nonpregnant values. The consistency of this fall, like that of the initial rise, indicates quite clearly that the elevation of SPI during pregnancy is a feature characteristic of pregnancy, not of the subjects of this particular study. The data are too scanty to permit precise definition of the course of the decline. Occasionally it was quite precipitate. In most instances it did not become evident until the second week postpartum, or even later. At first it was rapid, thereafter continuing, sometimes for a period of several weeks, at a more gradual rate. The possibility that these variations may be related to lactation deserves inquiry. When the decline was delayed, signs of overactivity of the thyroid remained in abeyance. The tolerance to high SPI appears to persist for an appreciable interval after the termination of pregnancy.

In addition to the subjects of Figure 1, 14 apparently normal women are still undelivered at the present date. Table I shows the duration of pregnancy to date in each case, the SPI values and the duration of the pregnancy at the time the SPI was measured. In all cases SPI was greater than 6.0 $\gamma$ per cent before the end of 16 weeks, usually far earlier. In one instance, IB, after 12 weeks it was 7.9 $\gamma$ per cent. Though it fell sharply to 4.0 $\gamma$ per cent at the end of 15 weeks, it had returned to 6.2 $\gamma$ per cent in another week and thereafter has remained elevated. This illustrates the fluctuations which were mentioned above. When MJ was first seen, after eight weeks of pregnancy, without any signs of hypothyroidism, her SPI was only 3.7 $\gamma$ per cent. After ten weeks it had risen only to 4.0 $\gamma$ per cent. Three weeks later, however, at the end of 13 weeks it had risen to 6.1 $\gamma$ per cent. After 21 weeks it had dipped slightly to 5.6 $\gamma$ per cent. Its further course has not been determined, but the patient is within six weeks of term. The woman appears to be one of those with a delayed rise. In two instances SPI was measured before pregnancy. In one, RR, it was above 6.0 $\gamma$ per cent and had not risen higher eight weeks after the last menstrual period. In the other it had risen from a prepregnancy concentration of 5.7 to 7.4 $\gamma$ per cent after six weeks of pregnancy. These cases lend support to the thesis that SPI normally rises comparatively early in pregnancy to 6 $\gamma$ per cent or more and remains elevated until delivery.

TABLE I

*Undelivered, apparently normal pregnant women*

| Patient | Duration of pregnancy* | Weeks after last menstrual period† | SPI |
|---|---|---|---|
| | *weeks* | | *$\gamma$ per cent* |
| C5279 | 38 | 28 | 8.6 |
| IB | 36 | 12 | 7.9 |
| | | 15 | 4.0 |
| | | 16 | 6.2 |
| | | 20 | 6.6 |
| | | 23 | 7.0 |
| | | 27 | 7.3 |
| | | 31 | 7.0 |
| | | 33 | 6.9 |
| C38229 | 19 | 7 | 11.9 |
| HC | 17 | 8 | 7.0 |
| MD | 22 | 8 | 8.6 |
| VD | 24 | 22 | 10.6 |
| BH | 37 | 3 | 7.7 |
| | | 5 | 6.0 |
| | . | 7 | 6.6 |
| | | 13 | 9.0 |
| | | 26 | 9.0 |
| MJ | 34 | 8 | 3.7 |
| | | 10 | 4.0 |
| | | 13 | 6.1 |
| | | 21 | 5.6 |
| PJ | 16 | 9 | 7.7 |
| | | 14 | 6.4 |
| MM | 26 | 19 | 7.1 |
| | | 25 | 7.0 |
| Re | 37 | 7 | 8.1 |
| RR | 42 | −22 | 6.3 |
| | | 8 | 6.2 |
| CT | 27 | −29 | 5.7 |
| | | 6 | 7.4 |
| | | 10 | 7.8 |
| CeT | 20 | 12 | 6.4 |

* The duration at the time the paper was written.
† A minus sign denotes that the observation was made at the indicated interval before conception.

TABLE II

*Complicated pregnancies*

| Patient | Weeks after last menstrual period* | SPI | Complication |
|---|---|---|---|
| | | *$\gamma$ per cent* | |
| B73578 | 20 | 8.7 | Diabetes insipidus |
| 63694 | 8 | 9.7 | Diabetes mellitus, Kimmelstiel-Wilson |
| | p 26 | 7.4 | |
| C2128 | 40 | 7.4 | Toxemia of pregnancy |
| C22464 | 11 | 7.5 | Multiple sclerosis |
| | p 1 | 6.6 | |
| 86295 | 17 | 6.9 | Toxemia of pregnancy, thrombophlebitis |
| C26972 | 40 | 6.0 | Toxemia of pregnancy |
| C15296 | 34 | 4.8 | Toxemia of pregnancy |
| | 40 | 4.8 | |
| 5911 | a 4 years | 4.1 | Diabetes |
| | 40 | 6.0 | |
| | p 1 | 4.6 | |
| 13258 | 30 | 12.1 | Toxemia of pregnancy |
| B77955 | 8 | 8.4 | Toxemia of pregnancy |
| B94664 | 8 | 7.0 | Toxemia of pregnancy |
| 31123 | 8 | 7.2 | Vascular disease |

* a denotes that the observation was made at the indicated interval before pregnancy; p denotes that the observation was made at the indicated interval after delivery.

*Abnormal pregnancy.* In addition to the patients with normal pregnancies, 12 pregnant women with complications of one kind or another, not involving the thyroid gland, were studied. The observations on these cases are shown in Table II. Of the 12 patients, eight had toxemias of pregnancy or vascular disease, two had diabetes, one with a Kimmelstiel-Wilson syndrome, one had diabetes insipidus and one multiple sclerosis. In spite of these complications, SPI fell in the range characteristic of pregnancy in all but one case, C15296. This patient was studied at the end of 34 weeks and again just before delivery. On both occasions SPI was 4.8 $\gamma$ per cent. What it may have been earlier or later is unknown. In two instances, 63694 and C22464, pregnancy was terminated: in one for a Kimmelstiel-Wilson syndrome, in the other for multiple sclerosis. In both SPI fell in the characteristic manner after pregnancy. In a patient with diabetes, 5911, SPI was measured before, during, and after pregnancy. The value during pregnancy was 1.9 and 1.4 $\gamma$ per cent greater than the values before and after pregnancy, respectively. These complications, therefore, appear not to modify the effects of pregnancy upon SPI.

There were two patients with ectopic pregnancies. One, three weeks after her last menstrual period, had an SPI of 4.0 γ per cent. Further observations could not be obtained. The second, after ten weeks of pregnancy, had an SPI of 8.3 γ per cent, which had fallen to 6.8 γ per cent 13 days after operation.

*Abortions.* In Table III are epitomized the records of 26 patients who aborted within the first 16 weeks of pregnancy. SPI was less than 6.0 γ per cent in 18 of the 26. This suggests that failure of SPI to rise during the first 16 weeks is conducive to abortion. Such an inference, however, must be drawn with reservations. In some instances the serum was drawn after bleeding or cramps had begun, occasionally on the day of the abortion. Under such circumstances it is impossible to tell whether the analysis was made before or after the termination of the pregnancy. Sometimes—for example, in Cases DP and MB—the abortion definitely preceded the measurement of

TABLE III

*Serum precipitable iodine of patients who aborted*

| Patient | SPI | Weeks* | Time of abortion† p last SPI | Thyroid‡ given | Remarks |
|---|---|---|---|---|---|
| | *γ per cent* | | | *gm./day* | |
| DB | 5.8 | 12 | 1 day | | |
| | 3.5 | p 6 | | | |
| EBr | 4.5 | 16 | 0 day | | |
| | 5.2 | p 22 | | | |
| EKe | 5.6 | E | 0 day | | |
| B68002 | 5.4 | 7 | 9 weeks | | |
| DP | 6.9 | 13 | −? day | | Abortion just before SPI |
| NP | 6.5 | 11 | | | |
| | 5.2 | p 52 | ? | | |
| C5510 | 7.0 | p | −1 day | | |
| ABl | 8.3 | 6 | 10 weeks | T | |
| BH | 2.7 | 7 | | | |
| | 4.7 | p 8 | ± 1 day | T | |
| MG | 5.4 | 8 | 3 days | Th | |
| B98775 | 4.6 | 15 | 1 day | Th | |
| DC | 5.8 | 7 | | T | May have aborted at time of first SPI |
| | 7.5 | 10 | | Th | |
| | 7.1 | 12 | 1 week | 0.24 | |
| RD | 4.0 | 5 | | Th | |
| | 7.0 | 9 | 4 weeks | 0.24 | |
| LJ | 4.7 | 12 | 2 weeks | Th | |
| HR | 7.6 | 7 | | 0.12 | Slight bleeding |
| | 6.8 | 12 | 2 weeks | 0.12 | |
| EBe | 4.7 | 6 | 3 weeks | Th | ? Hypothyroid |
| EI | 5.4 | 10 | 1 week | 0.12 | |
| BK | 7.0 | 7 | 1 week | 0.03 | |
| LL | 7.0 | 7 | 1 week | 0.06 | |
| HS | 4.3 | E | few days | 0.12 | |
| RBa | 6.5 | a 4 | | | |
| | 5.3 | 4 | few days | | |
| MB | 6.1 | 8 | −1 day | | |
| IB | 3.3 | 12 | few days | 0.18 | |
| VH | 3.8 | E | few days | | |
| MPo₁ | 7.3 | 13 | | 0.33 | Abortion threatened |
| | 8.5 | 16 | 1 day | 0.33 | |
| | 4.6 | p 4 | | | |
| MPo₂ | 2.8 | E | 9 days | 0.12 | |
| | 4.4 | p 2 | | | |
| EWi | 4.6 | a 9 | | | |
| | 5.2 | 9 | 4 weeks | | |
| | 4.6 | p 4 | | | |

* If not otherwise marked, indicates weeks after last menstrual period; a indicates weeks before conception; p indicates weeks after delivery. E indicates early in pregnancy, the exact time being unknown.

† A minus sign indicates that SPI was done after the abortion, 0 that it was done on the day of the abortion; ? that the precise interval is unknown.

‡ T indicates that thyroxine was given intravenously immediately after the SPI was done. Th indicates that thyroid was given at the first sign of abortion. The daily dose of thyroid is given when the drug was administered for an appreciable time.

SPI. In other cases, such as B68002 and AB1, the abortion occurred so long after the determination of SPI that it is impossible to relate the two. The SPI of B68002, at the end of seven weeks, was 5.4 $\gamma$ per cent. It is quite impossible to assert that nine weeks later, when she aborted, it had not risen above 6.0 $\gamma$ per cent. Conversely, AB1's SPI of 8.3 $\gamma$ per cent at the end of six weeks may have fallen before her abortion ten weeks later. In five cases (DB, EBr, NP, RBa and EWi) figures for SPI before or after pregnancy are available for comparison. In every case these are distinctly different. In two, DB and EWi, the SPI was higher in pregnancy, but below 6.0 $\gamma$ per cent. In a third, NP, it was 6.5 $\gamma$ per cent in pregnancy. In the remaining two, EBr and RBa, SPI appears to have fallen instead of rising during pregnancy. In no instance was the SPI outside of pregnancy below the normal range. In eight cases SPI was over 6.0 $\gamma$ per cent. Whether or not, therefore, a low value is incompatible with continuation of pregnancy, a high value does not insure against abortion. In seven cases thyroid or thyroxine, or both, were given at the time SPI was measured, because of bleeding, without preventing the abortion. In most of these abortion followed so rapidly that the thyroid can have had little effect. It is possible that some of these patients had aborted before the thyroid or thyroxine was given. DC, for example, was given thyroxine for bleeding after the seventh week. The bleeding stopped and SPI rose and remained high under continued treatment with thyroid. At the end of the 12th week she bled again. The Ascheim-Zondek test at this time proved to be negative. It seems highly probable that she actually aborted on the earlier occasion. RD, on the other hand, did not escape an abortion, despite the administration of enough thyroid to raise her SPI from 4.0 to 7.0 $\gamma$ per cent. Whether it remained at this level is uncertain, because the dose of thyroid was reduced from 0.24 to 0.12 gm. between the second determination and abortion. Several patients were already receiving variable amounts of thyroid when SPI was first measured. In four of these EI, HS, IB, and $MPo_2$, SPI was low in spite of the medication. In $MPo_2$, despite 0.12 gm. of thyroid daily, SPI was lower during pregnancy, 2.8 $\gamma$ per cent, than it was two weeks after abortion, 4.4 $\gamma$ per cent. Although these figures suggest that low SPI conduces to abortion, they permit no definite conclusions, chiefly because only single observations are available on subjects who did not receive thyroid. It is clear that administration of thyroid after bleeding has begun will not consistently prevent abortion, even if it raises SPI above 6.0 $\gamma$ per cent.

TABLE IV

*Patients who, having previously aborted (see Table III), had successful pregnancies*

| Patient | SPI | Weeks* | Thyroid given† | |
|---|---|---|---|---|
| | | | Before | After |
| | *$\gamma$ per cent* | | *gm./day* | *gm./day* |
| BH | 6.5 | 8 | 0 | 0 |
| | 5.9 | 10 | 0 | 0 |
| | 5.9 | 12 | 0 | 0 |
| MG | 8.7 | 13 | 0 | 0 |
| RBa | 6.1 | 4 | | |
| | 5.8 | 15 | 0.09 | 0.09 |
| MB | 5.9 | 6 | | 0.12 |
| | 5.9 | 10 | 0.12 | 0.18 |
| | 8.0 | 11 | 0.18 | 0.18 |
| | 7.1 | 18 | 0.18 | 0.12 |
| IB | 3.8 | 26 | 0.15 | 0.15 |
| MPo | 8.3 | 10 | 0.24 | 0.30 |
| | 10.0 | 16 | 0.30 | 0.30 |
| | 8.6 | 31 | 0.30 | 0.30 |

* Weeks after last menstrual period.

† *Before* indicates that the patient was receiving thyroid before the determination of SPI. *After* indicates that medication was begun after the determination of SPI.

In Table IV are presented the data from six patients who, having aborted in one pregnancy, successfully carried through another to delivery. The records during the pregnancy that ended in abortion have been given in Table III. The first two, BH and MG, had no complications nor special therapy during their successful pregnancies. Both had, however, far higher SPI's on the second occasions. The SPI of BH after seven weeks of her earlier pregnancy was 2.7 $\gamma$ per cent; in her successful pregnancy it was 6.5 $\gamma$ per cent at the end of eight weeks and subsequent figures after ten and 12 weeks were 5.9 $\gamma$ per cent. MG had SPI's of 5.4 and 8.7 $\gamma$ per cent, respectively. The comparison in this case is not so precise as it is in BH, because the analysis in the early pregnancy was made after eight weeks, while in the successful pregnancy it was made after 13 weeks. In both instances during the earlier pregnancy, after bleeding had begun, administration of thyroxine and thyroid failed to prevent abortion. The remaining four cases were treated with thyroid. Among these, RBa and MPo had higher SPI's in their successful pregnancies. The remainder were already receiving thyroid or were given thyroid when SPI was first measured. In every case but one, initial SPI's were greater than 5.0, and subsequent values never lower than 5.8 γ per cent. The exception, IB, is quite unique. The only determination, made after 26 weeks, was 3.8 γ per cent, when the patient was receiving daily 0.15 gm. of thyroid.

Forty-eight women, without evidence of hypothyroidism, successfully completed 51 pregnancies while receiving thyroid substance. This treatment was instituted for such diverse reasons that in the majority of cases it is impossible to determine whether or not it prevented abortions or contributed in any way to the well-being of the mother or fetus. Frequently it was begun before the first observation of SPI or even before the onset of pregnancy because of a history of previous abortions or infertility. So commonly is thyroid administered in pregnancy for various reasons without objective criteria of deficient thyroid function that its beneficial effect is almost impossible to evaluate. In ten of the 57 pregnancies abortion was threatened at one time or another, but pregnancy continued to term. These are presented in Table V. Bleeding or cramps occurred in eight of these during the first 16 weeks. Of these eight only four had SPI's of 6.0 γ per cent or less. In the other four SPI varied from 6.9 to 7.3 γ per cent. Thyroid was given to all eight immediately. Obviously SPI greater than 6.0 γ per cent does not confer protection against bleeding or cramps. But just as obviously it is impossible to assert that these patients would have aborted if thyroid had not been given. In two instances bleeding occurred after 20 and 21 weeks, respectively. In one the SPI had risen gradually from 4.8 γ per cent at seven weeks to 6.6 γ per cent at 20 weeks. The patient had received 0.06 gm. of thyroid daily up to this time, when bleeding began. She was immediately given 2 mg. of thyroxine intravenously. Three days later SPI was 18.1 γ per cent. The bleeding stopped. At the end of four more days when SPI had fallen to 12.8 γ per cent, she was given a continuing dose of 0.12 gm. daily, which was increased to 0.18 gm. at the end of 25 weeks. On this dose SPI fell to 7.8 γ per cent at the end of 33 weeks and to 5.8 γ per cent after delivery. The SPI of the second patient was 7.3 γ per cent at the end of 13 weeks, but six weeks later had dropped to 6.7 γ per cent in spite of the fact that she had meanwhile been given 0.12 gm. of thyroid daily. At the end of 21 weeks she began to bleed. Whether SPI had fallen further was not ascertained. The dose of thyroid was at once increased to 0.24 gm. Six weeks later, when SPI was 8.5 γ per cent, the dose was reduced to 0.12 gm. again because the patient complained of nervousness. With this one exception, possibly transient, at a late stage of pregnancy, after 16 weeks all values were 6.0 γ per cent or higher. In four of the subjects postpartum values were below 6.0 γ per cent, but within the normal range.

TABLE V

*Patients with threatened abortions*

| Patient | SPI | Weeks* | Thyroid given† | | Remarks |
|---|---|---|---|---|---|
| | | | Before | After | |
| | *γ per cent* | | *gm./day* | *gm./day* | |
| FB | 7.3 | 15 | 0 | 0.12 | Threatened abortion |
| | 6.4 | 20 | 0.12 | 0.18 | |
| | 8.5 | 30 | 0.18 | 0.18 | |
| | 4.1 | p 9 d. | 0‡ | | |
| ABu | 7.2 | 12 | | + | Threatened abortion |
| B12368 | 5.6 | 15 | 0 | 0.06 | Threatened abortion |
| | 7.4 | 29 | 0.09 | 0.09 | |
| | 4.1 | p 19 | 0‡ | | |
| CG | 5.5 | a 11 | | | |
| | 4.8 | 7 | 0.06 | 0.06 | |
| | 5.1 | 11 | 0.06 | 0.06 | |
| | 6.6 | 20 | 0.06 | T 2 mg. | Slight bleeding and labor pains |
| | 18.1 | 20 | | 0.12 | 3 days later |
| | 12.8 | 21 | 0.12 | 0.18 | |
| | 10.0 | 25 | 0.18 | 0.18 | |
| | 7.8 | 33 | 0.18 | 0.18 | |
| | 5.8 | p 15 | 0.18 | 0.12 | |
| | 5.8 | p 28 | 0.12 | 0.12 | |
| AL | 6.0 | 5 | 0 | T 1 mg. | Severe cramps |
| | 5.4 | 12 | 0.06 | 0.12 | |
| EM | 4.2 | 6 | 0 | 0.18 | Slight bleeding |
| | 6.4 | 13 | 0.18 | 0.18 | |
| | 6.3 | 22 | 0.18 | 0.18 | |
| | 4.5 | p 9 | 0‡ | | |
| | 5.0 | p 30 | 0‡ | | |
| MM (1) | 7.3 | 13 | 0 | 0.12 | |
| | 6.7 | 19 | 0.12 | 0.12 | Spotting at 21 weeks |
| | 8.5 | 27 | 0.24 | 0.12 | |
| | 5.8 | 32 | 0.12 | 0.12 | |
| (2) | 7.2 | 11 | 0.12 | 0.12 | Second pregnancy |
| PR | 6.9 | 8 | 0.06 | 0.06 | Bleeding, repeated at 10 weeks |
| | 6.9 | 12 | 0.06 | 0.06 | |
| 55146 (1) | 5.1 | 8 | 0 | T 3 mg. | Threatened abortion |
| | 6.8 | 17 | 0.12 | 0.12 | |
| | 6.0 | 38 | 0.12 | 0.06 | |
| (2) | 5.7 | 9 | 0 | 0 | |
| | 6.5 | 14 | 0 | 0.12 | |
| | 7.3 | 18 | 0.12 | 0.12 | |
| BT | 6.9 | 11 | 0 | 0.12 | Bleeding |

* a indicates before pregnancy, p indicates after delivery.

† *Before* indicates that the patient was already receiving thyroid. *After* indicates that thyroid was begun after the measurement of SPI.

‡ No thyroid given after delivery.

In these respects, therefore, whether administration of thyroid contributed to the prevention of abortion or not, SPI followed the course characteristic of normal pregnancy.

*Effects of administration of dried thyroid or thyroxine.* In Table VI are presented the records of 23 women who were given thyroid or larger doses of thyroid specifically because SPI was low in the early stages of pregnancy. All delivered at term. In the majority of those who had repeated examinations, SPI rose above 6.0 $\gamma$ per cent. The number of prolonged studies is, however, small. In four instances (GM, EWa, EsB and RBl), SPI was less than 6.0 $\gamma$ per cent between 16 and 20 weeks in spite of thyroid. Two of these probably represent transitory drops: GM to 5.2 $\gamma$ per cent at 17 weeks, and EWa to 4.7 $\gamma$ per cent at 18 weeks. The latter had an SPI of 7.7 $\gamma$ per cent at 29 weeks. In this instance SPI fell from 5.3 $\gamma$ per cent at 13 weeks to 4.7 $\gamma$ per cent at 18 weeks in spite of 0.18 gm. of thyroid daily. A single observation on EsB at 17 weeks, while she was receiving 0.12 gm. of thyroid daily, was 5.0 $\gamma$ per cent. The remaining determination on RBl, at 19 weeks, while she was receiving 0.12 gm. of thyroid, was 5.8 $\gamma$ per cent, practically indistinguishable from 6.0 $\gamma$ per cent. In one patient, BE, SPI of 5.6 $\gamma$ per cent at 16 weeks, without medication, fell to 3.7 $\gamma$ per cent at 24 weeks on 0.12 gm. of thyroid. This suggests that she disposed of exogenous thyroid with great facility. Such sporadic low values in the later stages of pregnancy have been noted earlier. In most instances SPI appears to be highly responsive to the administration of thyroid. It is not, however, possible in most of these to distinguish the effects of thyroid from the influence of pregnancy itself. The SPI of ACo, for example, before pregnancy, when she was not receiving thyroid, was 7.2 $\gamma$ per cent. In her first pregnancy, for no clear reason, she was given 0.18 gm. of thyroid at nine weeks. Without any change of this dose, SPI was successively 7.5, 8.5, and 9.4 $\gamma$ per cent at 13, 19, and 32 weeks respectively. This patient is remarkable in certain other respects. During the course of the year succeeding her delivery, without thyroid, she had an abortion. In a subsequent pregnancy, which is recorded in the Table, her SPI at six weeks was only 4.7 $\gamma$ per cent. On 0.18 gm. of thyroid it rose to 5.9 $\gamma$ per cent after a week and to 8.3 $\gamma$ per cent at 17 weeks, approximately the same as its concentration at the same stage of her first pregnancy. GGl was given 0.24 gm. of thyroid daily at seven weeks, when her SPI was 5.6 $\gamma$ per cent. Without any change of dose, the SPI rose

TABLE VI

*Patients who received thyroid because of low SPI*

| Patient | SPI | Weeks* | Thyroid given† | | Remarks |
|---|---|---|---|---|---|
| | | | Before | After | |
| | *$\gamma$ per cent* | | *gm./day* | *gm./day* | |
| ACo$_1$ | 7.2 | a 46 | 0 | 0 | |
| | 7.5 | 13 | 0.18 | 0.18 | |
| | 8.5 | 19 | 0.18 | 0.18 | |
| | 9.4 | 32 | 0.18 | 0.18 | |
| ACo$_2$ | 4.7 | 6 | 0 | 0.18 | |
| | 5.9 | 7 | 0.18 | 0.18 | |
| | 8.3 | 17 | 0.18 | 0.18 | Thyroid reduced to 0.06 after 28 weeks |
| GGl | 5.6 | 7 | 0 | 0.24 | |
| | 6.4 | 9 | 0.24 | 0.24 | |
| | 10.9 | 12 | 0.24 | 0.24 | |
| | 8.3 | 15 | 0.24 | 0.24 | |
| | 7.0 | 25 | 0.24 | 0.24 | |
| | 6.9 | 32 | 0.24 | 0.24 | |
| | 5.2 | p 16 | 0 | | No thyroid after delivery |
| EH | 5.6 | a 5 | 0 | | |
| | 4.6 | 7 | 0 | 0.18 | |
| | 7.6 | 11 | 0.18 | 0.18 | |
| | 6.8 | 15 | 0.18 | 0.18 | |
| | 7.6 | 25 | 0.18 | 0.18 | |
| B95180 | 5.7 | 15 | 0 | 0 | |
| | 6.6 | 22 | 0.18 | 0.24 | |
| | 6.9 | 36 | 0.24 | 0.24 | |
| | 4.6 | p 38 | 0 | | No thyroid after delivery |
| GM | 5.2 | 4 | 0 | 0.12 | |
| | 5.9 | 12 | 0.12 | 0.12 | |
| | 5.2 | 17 | 0.12 | 0.12 | |
| MR | 5.8 | 6 | 0 | 0.12 | |
| | 9.7 | 12 | 0.12 | 0.12 | |
| C23634 | 4.2 | a 1 | 0 | 0 | |
| | 5.2 | 8 | 0 | 0.18 | |
| | 7.1 | 19 | 0.18 | 0.18 | |
| EV | 5.3 | a 12 | 0 | 0 | |
| | 4.0 | 6 | 0 | 0 | |
| | 5.6 | 7 | 0 | 0.24 | |
| | 7.5 | 7 | 0.24 | 0.24 | 3 days later |
| | 10.0 | 13 | 0.24 | 0.18 | |
| EWa | 5.3 | 13 | 0 | 0.12 | |
| | 6.5 | 15 | 0.12 | 0.18 | |
| | 4.7 | 18 | 0.12 | 0.18 | On only 0.12 for 1 week |
| | 7.7 | 29 | 0.18 | 0.18 | |
| | 5.4 | p 8 | 0 | | No thyroid after delivery |
| JW | 5.9 | 10 | 0 | 0.18 | |
| MC | 3.2 | 6 | 0.12 | 0.30 | |
| | 6.9 | 15 | 0.30 | 0.30 | |
| MPe | 3.7 | 6 | 0 | 0.18 | |
| EsB | 5.0 | 17 | 0.12 | 0.12 | |
| RBl | 5.7 | 5 | 0 | 0.12 | |
| | 5.8 | 19 | 0.12 | 0.12 | |
| JB | 5.8 | 6 | 0 | 0.12 | |
| BE | 5.6 | 16 | 0 | 0.09 | |
| | 3.7 | 24 | 0.09 | 0.09 | |
| TI | 5.6 | 6 | 0.12 | 0.24 | |
| | 8.7 | 16 | 0.24 | 0.12 | |
| SJ | 4.9 | 6 | 0 | 0.18 | |
| CK | 5.2 | 8 | 0 | 0.12 | |
| ML | 5.4 | 13 | 0 | 0.18 | |
| | 7.3 | 23 | 0.18 | 0.12 | |
| JP | 5.8 | 13 | 0 | 0.09 | |
| | 8.3 | 20 | 0.09 | 0.09 | |
| KW | 4.2 | 8 | 0.09 | 0.09 | |
| GGe | 5.5 | 7 | 0.12 | 0.12 | |
| | 6.9 | 13 | 0.12 | 0.12 | |

* a indicates before pregnancy, p indicates after delivery.

† *Before* indicates that the patient was already receiving thyroid. *After* indicates that thyroid was begun after the measurement of SPI.

TABLE VII

*Patients who received thyroid and delivered normally, although no SPI lower than 6.0 γ per cent was observed*

| Patient | SPI | Weeks* | Thyroid given† | | Remarks |
|---|---|---|---|---|---|
| | | | Before | After | |
| | *γ per cent* | | *gm./day* | *gm./day* | |
| JK | 6.2 | 18 | | 0.25 | |
| | 6.8 | 23 | 0.25 | 0.25 | |
| HL | 7.6 | 19 | | | |
| | 7.6 | 26 | | | |
| | 5.5 | 31 | 0.12 | 0.12 | |
| | 4.8 | 32 | 0.12 | 0.12 | |
| DM | 6.0 | 30 | 0.06 | 0.06 | |
| | 7.2 | p 30 | 0.06 | 0.06 | |
| | 6.4 | p 100 | 0.06 | | |
| HP | 5.1 | a 17 | | | |
| | 6.3 | 7 | 0 | 0.18 | |
| | 9.9 | 9 | 0.18 | 0.18 | |
| | 5.8 | 20 | 0.18 | 0.18 | |
| CP | 6.0 | 11 | 0 | 0.12 | |
| | 5.9 | 16 | 0.24 | 0.24 | 0.24 begun after 14 weeks |
| | 6.3 | 20 | 0.24 | 0.24 | |
| HT | 6.1 | 9 | 0 | 0.18 | |
| | 7.1 | 12 | 0.18 | 0.18 | |
| | 6.6 | 16 | 0.18 | 0.18 | |
| C24722 | 7.3 | 30 | 0.18 | 0.18 | Thyroid started at onset of pregnancy |
| | 6.4 | 37 | 0.18 | 0.18 | |
| EKo | 6.3 | 6 | 0 | 0.06 | 0.18 begun after 11 weeks |
| | 9.2 | 18 | 0.18 | 0.18 | |
| MN | 4.9 | a 124 | 0.06 | 0.06 | |
| | 6.5 | 17 | 0.06 | 0.12 | |
| | 7.8 | 20 | 0.12 | 0.12 | |
| | 8.3 | 27 | 0.12 | 0.09 | |
| CR | 6.1 | 10 | 0.12 | 0.12 | |
| | 5.5 | 13 | 0.12 | 0.24 | |
| RR | 6.3 | a 22 | | | |
| | 6.2 | 8 | 0.12 | 0.18 | |
| HB | 7.3 | 12 | 0.06 | 0.06 | |
| LE | 7.3 | 8 | 0 | 0.06 | |
| MPl | 6.6 | 8 | 0 | 0.09 | |
| | 6.4 | 19 | 0.09 | 0.03 | |
| DS | 6.7 | 13 | 0 | 0.06 | |

* If not otherwise marked, indicates weeks after last menstrual period; a indicates weeks before conception; p indicates weeks after delivery.

† *Before* indicates that the patient was already receiving thyroid. *After* indicates that thyroid was begun after the measurement of SPI.

to a peak of 10.9 γ per cent at 12 weeks and thereafter gradually declined to 6.9 γ per cent at 32 weeks. After delivery, when thyroid had been discontinued, it fell still further, to 5.2 γ per cent. This suggests that, although thyroid may accelerate the initial rise of SPI, thereafter, on a constant dose, SPI follows the course usual in pregnancy. In spite of the fact that doses of from 0.18 to 0.30 gm. of thyroid were given to these subjects over long periods, SPI did not exceed the concentrations ordinarily encountered in pregnancy nor were symptoms or signs of hyperthyroidism encountered. This may denote that the pregnant woman is tolerant of the thyroid hormone or able to dispose of it with unusual facility. It must be recognized, however, that a proportion of normal women have a similar tolerance (10, 11).

If, as other evidence suggests, failure of SPI to rise is incompatible with the continuation of pregnancy, the data of Table VI suggest that administration of thyroid in generous amounts when SPI is low may prevent abortion. This would imply that the increment of SPI in pregnancy is composed of thyroid hormone. To verify this it is necessary, however, to prove more definitely that abortion is a regular consequence of a persistent low SPI. This requires the study of a larger number of untreated subjects in the early weeks of pregnancy.

Fifteen women who delivered normally were given thyroid in spite of the fact that their SPI's were 6.0 γ per cent or higher at the first observations during pregnancy, usually because of histories of previous infertility or abortions. The data from these cases are presented in Table VII. In no instance did the thyroid drive SPI above the normal range for pregnancy. In two, in fact, SPI fell after the administration of thyroid. In HL the low values were observed in the last trimester of pregnancy. In this case thyroid was not given until the end of 27 weeks. In HP, after rising from 6.3 to 9.9 γ per cent between seven and nine weeks on 0.18 gm. of thyroid, SPI dropped to 5.8 γ per cent at the end of 20 weeks. Its subsequent course is unknown.

In both these last series shown in Tables VI

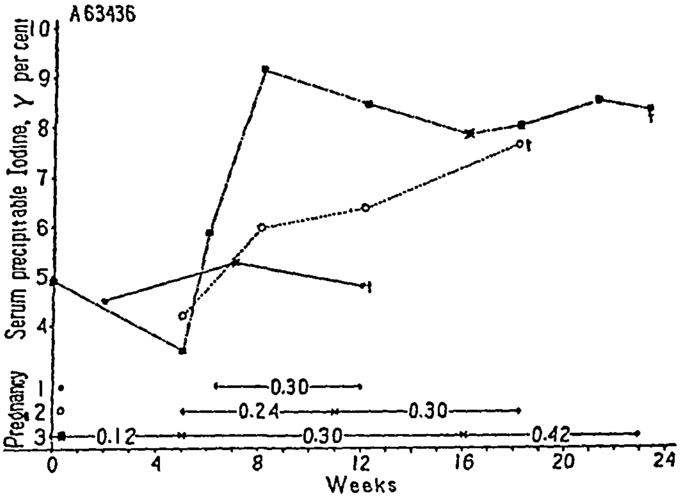

FIG. 2. THE COURSE OF SPI OF A SUBJECT IN THREE PREGNANCIES THAT ENDED IN ABORTION

The daily dose of thyroid, in grams, given during each pregnancy, together with the duration of its administration, is shown by the figures and arrows below the curves. Crosses indicate bleeding or cramps; daggers represent abortion.

and VII, as in previous series, SPI before and after pregnancy, when it was measured, lay within the normal nonpregnant range.

That thyroid may not be successful in preventing abortion, even if it is given in large doses before any signs or symptoms appear, is illustrated by the woman whose record is illustrated in Figure 2. In her first pregnancy, at two weeks and seven weeks SPI's were 4.5 and 5.3 $\gamma$ per cent, respectively. At this time, because of bleeding, she was given 0.30 gm. of thyroid daily. In spite of this she aborted and curettage was necessary at 12 weeks. Three months later she again became pregnant. After five weeks, because SPI was only 4.2 $\gamma$ per cent, she was given 0.24 gm. of thyroid. At eight weeks SPI had risen to 6.0 $\gamma$ per cent. Two weeks later the dose of thyroid was raised to 0.30 gm. Although SPI was 7.7 $\gamma$ per cent at 18 weeks, she again aborted. Three months later, without thyroid, SPI was 4.9 $\gamma$ per cent, a normal value. Nevertheless, she was given 0.12 gm. of thyroid daily. Her last menstrual period occurred two days later. At the end of five weks SPI was only 3.5 $\gamma$ per cent, although the dose of thyroid had been increased to 0.24 gm. three days earlier. It was immediately raised to 0.30 gm. In another two weeks SPI was 9.2 $\gamma$ per cent. In spite of the fact that it did not fall below 8 $\gamma$ per cent, uterine contractions began at 16 weeks. These diminished after the dose of thyroid was increased to 0.42 gm., but she aborted after 23 weeks. In this case the rise of SPI in every pregnancy was delayed. In fact, in every instance it was lower than the only value obtained in the nonpregnant state. This was within normal limits, an indication that the subject had no thyroid deficiency. Nevertheless, large doses were required to raise SPI to characteristic pregnancy values. Even when thyroid was begun before pregnancy and relatively early elevation of SPI was achieved, abortion was not prevented. As the intensity of thyroid therapy was increased and as it was initiated at an earlier date, there was a suggestion that pregnancy was prolonged. In this case there appears to be some inherent obstacle to the conduct of normal pregnancy that is associated with low SPI. This can be ameliorated, but not abolished, by administration of enough thyroid at an early date to raise SPI to concentrations appropriate for pregnancy.

TABLE VIII

*Premature deliveries*

| Patient | SPI | Weeks* | Thyroid given† | | Remarks |
|---|---|---|---|---|---|
| | | | Before | After | |
| | *$\gamma$ per cent* | | *gm./day* | *gm./day* | |
| KA | 8.8 | 25 | 0.15 | 0 | Spotting for 6 days; delivery 2 days after SPI. |
| 19373 | 6.5 | 17 | 0 | 0 | Slight bleeding 4 weeks earlier; delivery at 25 weeks. |
| VH | 5.1 | 6 | 0.06 | 0.21 | |
| | 8.2 | 12 | 0.21 | 0.21 | Delivery at 36 weeks |
| B59643 | 7.7 | 22 | 0 | 0 | Delivery at 35 weeks |
| MRo | 9.9 | 16 | 0 | 0 | Delivery at 36 weeks |

* Weeks after last menstrual period.

† *Before* indicates that the patient was already receiving thyroid. *After* indicates that thyroid was begun after the measurement of SPI.

Similar conditions may obtain in those subjects reported above who aborted in spite of normal SPI.

*Premature deliveries.* Attention has been called frequently to occasional low values occurring late in pregnancy. It has been impossible to attach any significance to these. Furthermore, to date no relation has been discovered between "premature" deliveries in the latter months of pregnancy and SPI. The data on this point are, however, necessarily scanty. Five patients who received the diagnosis of premature delivery are presented in Table VIII. The first two do not properly belong in this category because both delivered at 25 weeks. The first, KA, just before delivery, on 0.15 gm. of thyroid daily had an SPI of 8.8 $\gamma$ per cent. The second, 19373, at 13 weeks bled slightly; but at 17 weeks had not aborted and had an SPI of 6.5 $\gamma$ per cent. She went into labor, however, and delivered at 25 weeks. The remaining three patients delivered at 35 or 36 weeks. One of them, VH, was given 0.06 gm. of thyroid before pregnancy because her SPI was only 3.8 $\gamma$ per cent, a low normal figure. At six weeks, because SPI had risen only to 5.1 $\gamma$ per cent, the dose of thyroid was increased to 0.21 gm. daily. The SPI at 12 weeks had risen to 8.2 $\gamma$ per cent. Although the thyroid was continued, she had a premature delivery after 36 weeks. The SPI's of the remaining two appear to have risen to proper pregnancy concentrations. Their pregnancies were uneventful until their deliveries at 35 and 36 weeks respectively. Neither received thyroid. It is impossible in any

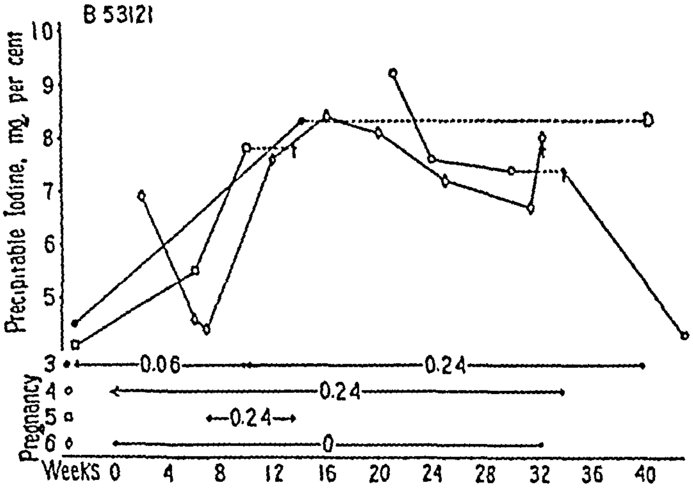

FIG. 3. THE COURSE OF SPI DURING FOUR PREGNANCIES OF A SUBJECT WITH A TENDENCY TO PREMATURE DELIVERY

The general construction of the chart is similar to that of Figure 2. D represents normal delivery.

of these cases to be certain that SPI did not fall later in the course of pregnancy.

One patient, whose record is illustrated in Figure 3, merits especial consideration. At the age of 22, because of a basal metabolism of − 23, she was given 0.0125 gm. of thyroid daily. In 1939, when her basal metabolism was found to be − 12, she was given 0.06 gm. of thyroid daily. On neither occasion did she have symptoms of hypothyroidism. In November, 1942, her right tube was removed for a tubal pregnancy. In February, 1946, she had an abortion at three months. July 22, 1946, while on 0.06 gm. of thyroid daily, her SPI was 4.5 γ per cent. October 24 she became pregnant. Shortly after this the dose of thyroid was increased to 0.24 gm. daily. She had an uncomplicated pregnancy, except for slight bleeding in the first months at the times of her menstrual periods, and was delivered on July 23, 1947, at term, of a normal child. On November 10 of that year she again became pregnant. Thyroid 0.24 gm./day was begun on December 27. Again she bled slightly in the first months at the time of her normal menstrual periods; but otherwise her pregnancy proceeded normally until July 4, 1948, when she went into labor and was delivered of a premature child who died two days later. Thyroid medication was discontinued after delivery. SPI's, 38 and 93 days after delivery, were 4.3 and 4.1 γ per cent, respectively. February 23, 1949, she again became pregnant. After six weeks her SPI was 5.5 γ per cent. She was again given 0.24 gm. of thyroid daily. Four weeks later her SPI was 7.8 γ per cent. Nevertheless she had a spontaneous abortion at the end of 13 weeks. On July 27 she once more became pregnant. On this occasion no thyroid was given. SPI was 6.9 γ per cent only two weeks after her last menstrual period. Although it fell to 4.6 and 4.4 γ per cent at six and seven weeks, it rose after this spontaneously and remained well above 6.0 γ per cent until the end of 32 weeks when she bled profusely. Despite administration of 2 mg. of thyroxine intravenously, she was delivered of a dead fetus within 24 hours, with evidence of premature separation of the placenta. From the values of SPI when she was neither pregnant nor receiving thyroid, as well as the absence of symptoms and signs, the patient was evidently not hypothyroid. There is no evidence that administration of thyroid altered the course of her pregnancies. The fact that one pregnancy went to term while she was receiving thyroid seems to be purely adventitious. Without thyroid, the last, her sixth pregnancy, proceeded quite as long as did the fourth in which she received thyroid.

## DISCUSSION

That SPI rises during pregnancy appears to have been established. This rise is attended by no clinical evidence of excessive activity of the thyroid gland. It occurs long before the increase of basal metabolism that accompanies pregnancy. The latter also presumably denotes no increased activity of the thyroid, but seems rather to be related to the need for extra oxygen created by the enlargement of the uterus and the growth of the fetus.

The almost complete absence of SPI's below 6.0 γ per cent after 16 weeks suggests that concentrations above this are, in normal persons, necessary for the successful completion of pregnancy. At the left of Figure 1 the limits of variation of SPI in various conditions are defined. It is evident that in a group of nonpregnant women of this size the majority of points would lie between 4 and 6 γ per cent, an area that is almost empty, and that there would be only an occasional spot above 7 γ per cent, which is the most thickly populated area of the figure. In the period before 12 or 16 weeks there are more points in the normal range. If all pregnant women were included, not only those who completed pregnancy without medication, the area between 4 and 6 γ per cent in the first weeks of pregnancy would be fuller. From the paucity of points below 6 γ per cent after 16 weeks, the fact that the SPI of non-pregnant women most frequently lies in this area, *and the greater frequency of such points in the* earlier weeks of pregnancy, it may be inferred that before the end of this period SPI regularly attains a concentration of 6 γ per cent or more and that when it fails to rise to this level abortion occurs.

In the patients who aborted and those who received thyroid substances, as in the normal pregnancies, there is the same tendency for SPI in pregnancy to lie above 6.0 γ per cent after 16 weeks. This is best illustrated in Figure 4 which includes all observations on patients who were delivered at term. A cross indicates that the subject was receiving thyroid, a dot that she was taking no medication. The paucity of points in the area below 6.0 γ per cent after 16 weeks is again evident. Of the total number of observations only ten are found in this area. Of these, five are greater than 5.0 γ per cent.

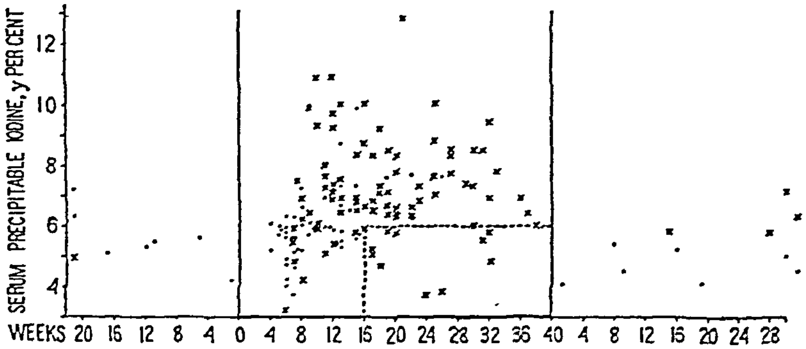

FIG. 4. THE SPI OF ALL SUBJECTS WHO RECEIVED THYROID AND SUCCESSFULLY COMPLETED PREGNANCY

Dots indicate that the subject had not received thyroid, crosses that she had received thyroid before the observation. The period of pregnancy is defined by the vertical lines. The area to the left represents time before, that to the right time after, pregnancy.

There is, however, in contrast to the series of normal pregnancies reported in the preceding paper, a larger aggregation of values below 6 γ per cent in the early weeks of pregnancy. This would be still greater if the observations in Table III from the subjects who aborted were included. The incidence of low points also seems to diminish as pregnancy advances to 16 weeks. SPI, therefore, in a considerable number of women, remains low in the early weeks of pregnancy. Some of the later points below 6 γ per cent represent transient fluctuations. However, the concentration 6.0 was selected somewhat arbitrarily because the great majority of observations lay above this concentration. It would be surprising indeed if there were not some overlap in the zone of differentiation. The two extremely low figures represent single observations on two patients. What the SPI may have been at other times in the course of pregnancy is unknown. On the whole the data support the earlier hypothesis that if pregnancy is to be completed, SPI is not consistently below about 6.0 γ per cent after pregnancy has proceeded approximately 16 weeks. This would mean that SPI regularly achieves this level in the course of the first 16 weeks in successful pregnancies. This suggests, but does not necessarily imply, that failure of SPI to rise in this period conduces to abortion. Definitive proof that this is the case must depend upon the intensive study of a large group of subjects during early pregnancy without medication.

In the small series thus far collected, early miscarriage appears to be more common in those with low SPI. An SPI above 6.0 γ per cent, however, obviously does not preclude abortion. If failure of SPI to rise does predetermine abortion, the question arises whether there is a critical time and a critical concentration. Sometimes

SPI exceeds 6.0 $\gamma$ per cent as early as the diagnosis of pregnancy can be established. In other cases it remains below this concentration until the period of 16 weeks is almost completed. The majority of miscarriages occur earlier than this. If low SPI is correlated with the cause, rather than the incidence, of miscarriage, why do some subjects tolerate low values longer than others do? Are those who do miscarry inherently incapable of raising their SPI's or would their SPI's eventually rise if they escaped miscarriage?

Failure of SPI to rise in those that miscarry may be an indication that there is a defect in the ovum or its implantation of such a kind that the stimulus that causes SPI to rise is lacking. In those in which SPI rises late, but abortion is avoided, this stimulus may be only partly lacking, or the defect may lie not in a deficient stimulus, but in a deficient or delayed reaction of the mechanism responsible for the rise of SPI, presumably the secretory activity of the thyroid gland or the utilization of the thyroid hormone, or both. In this case SPI would be little more than a symbol. Miscarriages would be associated with low SPI, but the latter would not necessarily determine a miscarriage. Such a hypothesis seems to be consistent with most of the facts presented. It gains some support from the cases in which the patient who miscarried in one pregnancy with low SPI had a subsequent successful pregnancy when SPI rose early (cases BH and MG, Tables III and IV). It might partly explain the consistent failure to complete pregnancies displayed by A63436 (Figure 2). It would not, however, account so well for the relative absence of low values after 16 weeks or the apparent prolongation of pregnancy by thyroid in A63436.

This hypothesis would relegate the elevation of SPI and the thyroid hormone to a derivative or adventitious position. Alternatively, completion of pregnancy may depend on the proper reactivity of the mechanism which causes SPI to rise, presumably increased secretory activity of the thyroid gland, with diminished or increased utilization of thyroid hormone. If the first hypothesis is correct, the common practice of administering thyroid to prevent abortion, which originated on a purely empirical basis, would be of no value. These two hypotheses are not, of course, mutually exclusive. It may be that both stimulus and reactivity of the thyroid gland must be adequate. The present study is intended in part to throw some light on these questions.

It is clear from the data of Table III that the administration of thyroid, or even thyroxine, in massive doses, will not regularly prevent abortion after symptoms have begun. In most instances, however, there can be no assurance that abortion was not already an accomplished fact before the thyroid or thyroxine was given. The generally higher values of SPI in the successful pregnancies of Table IV, as contrasted with those in the abortive pregnancies of the same individuals in Table III, suggest that a high SPI is advantageous and that administration of thyroid tends to raise SPI. The patients in Table V might be cited as more direct evidence of the beneficial effect of thyroid. In this group completion of abortion may have been prevented by timely administration of thyroid. In five of the ten pregnancies, medication was followed by a distinct rise of SPI. In three of the five, SPI at the onset of cramps or bleeding was lower than 6 $\gamma$ per cent. The total number of cases is, however, small and the number of controls who, under similar circumstances, did not receive thyroid is smaller. In Tables VI and VII is a goodly array of women who, having received thyroid, completed pregnancy successfully without symptoms of miscarriage at any time. The greater proportion of these (those in Table VI) were given thyroid because SPI was low. In most of those in which further observations were made, SPI rose in response to the medication, but repeated observations were all too few and again adequate controls are lacking. The data on the whole suggest, but certainly do not prove, that administration of thyroid prevents abortion.

Attention has been called to the incidence of abortions with SPI above 6 $\gamma$ per cent. If low SPI does promote abortion, these cases can not be summarily dismissed as belonging to a different category. In normal pregnancy some women carry higher SPI than others. This may be a necessary provision. In only one of the patients of this series, in addition to A63436 (Figure 2), did abortion or threatened abortion occur when SPI was 7.5 $\gamma$ per cent or higher. It may be more than a coincidence that the points below 6 $\gamma$ per cent after 16 weeks in Figure 4 are all derived from patients who were receiving thyroid. If the increment of SPI in pregnancy is composed of thyroid hormone, its significance still remains to be elucidated. It could mean diminished utilization of hormone, though this seems highly unlikely. It is generally held, from morphological studies, that secretory activity of the thyroid is increased and, as a corollary, that this is a response to an increased demand for the hormone to meet increased utilization. An increased supply would then be the prime necessity. With the administration of exogenous thyroid the tissues might be able to derive the necessary hormone from the circulation by more efficient extraction with a lower concentration in the serum.

Much of this discussion and the data on which it is based tend to pose rather than to answer questions as to the clinical value and significance of SPI in pregnancy. The data may, however, throw some light on the nature of the increment in pregnancy. Values of SPI as high as those encountered in pregnancy are regularly associated with symptoms of hyperthyroidism in nonpregnant women. This seems to denote either that the pregnant woman tolerates a greater concentration of hormone in her serum or that the increment is not composed of thyroid hormone. In many of the cases in this series, SPI was rapidly raised by administration of thyroid or thyroxine to levels characteristic of hyperthyroidism without appearance of symptoms. Presumably these increments of iodine were hormonal. Pregnancy does, therefore, appear to confer a tolerance for circulating thyroid hormone. In a few instances in which SPI was suddenly raised to unusually high concentrations it tended to decline to more moderate concentrations as pregnancy advanced. Whether the endogenous supply was reduced, utilization accelerated, or removal by other channels increased, it is impossible as yet to say. It is noteworthy that on the whole, regardless of the dose of thyroid, the concentration of SPI, with the exception of the temporary shoots mentioned above, tended to establish itself in the range characteristic of pregnancy.

This tolerance appears to differ somewhat from that displayed by nonpregnant women, in which the concentration of SPI does not rise, or, after a preliminary rise, recedes to the normal range (10). In most of the present series in which SPI was measured postpartum, thyroid was discontinued after delivery. In the two cases in which it was continued, it was found to be in the normal nonpregnant range. In one it fell after delivery. This strengthens the impression that SPI is sustained by pregnancy. Whether the increment of SPI is, like the greater proportion of the normal SPI, composed of thyroid hormone cannot be decided from these studies. It does behave like the thyroid hormone in attaching itself to the serum proteins. Furthermore, it is extracted by butanol (12, 13).

## CONCLUSIONS

The serum precipitable iodine has been determined on one or more occasions during pregnancy in 46 women without complications or signs of overactivity or underactivity of the thyroid gland who delivered at term. In only three instances was SPI observed below 6.0 γ per cent after 16 weeks of pregnancy. In nine patients with pregnancies complicated by conditions other than abnormal activity of the thyroid, SPI with one exception lay at the same high levels. This is also true of 14 patients still undelivered but with apparently normal pregnancies, well advanced. In those patients studied before and after pregnancy, SPI is found to rise early in pregnancy and to fall shortly after delivery.

The precipitable iodine of serum (SPI) has been measured in a series of euthyroid pregnant women who aborted or who received thyroid substance to prevent abortion. There is some evidence that if SPI does not rise above about 6 γ per cent during the first 16 weeks, pregnancy is unlikely to proceed to term and that abortion is more common in those women whose SPI's do not rise relatively early in pregnancy. Administration of generous amounts of thyroid substance accelerates the rise of SPI. The cases reported suggest that, if thyroid is given early enough, it may diminish the tendency to abortion. A larger body of controls is required to establish these points.

If thyroid is given to the pregnant woman, the curve of SPI tends to follow a course similar to that of the untreated pregnant woman. The implications of this fact with respect to the nature and significance of the increment of SPI in pregnancy are discussed.

ACKNOWLEDGMENTS

Thanks must be given to Drs. Irving Friedman and Milton S. Godfried for their generosity in permitting the use of data derived from their patients, and in making their records available; and to Dr. Herbert Thoms, Professor of Obstetrics and Gynecology, and his staff for their cooperation.

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# THE PRECIPITABLE IODINE OF SERUM IN PREGNANCY COMPLICATED BY DISORDERS OF THE THYROID[1]

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(Submitted for publication August 2, 1950; accepted, November 6, 1950)

In a series of papers (1–3) it has been shown that the precipitable iodine of the serum (SPI) rises in early pregnancy and remains elevated until delivery, thereafter returning to its usual concentration after a variable, but usually short, interval. Although SPI may rise to concentrations usually associated in nonpregnant women with hyperthyroidism, in pregnancy these rises are accompanied by no marks of excessive thyroid activity. In a large series of subjects without disorders of the thyroid, SPI was regularly below 7.8 $\gamma$ per cent, in hyperthyroid subjects it was regularly above 7.2 $\gamma$ per cent. Between these two points there was slight overlapping (4). In normal pregnancy, however, the majority of values lie above 7.2 $\gamma$ per cent, with some as high as 10.0 $\gamma$ per cent (3). The evidence indicates that failure of SPI to attain a concentration of about 6.0 $\gamma$ per cent or higher within the first 16 weeks of pregnancy predisposes to miscarriage, and suggests that administration of active thyroid substances to women with low SPI early in pregnancy may avert miscarriage (3).

The significance of the elevated SPI is quite obscure. This increment is presumably composed of thyroid hormone. If it is hormone it still remains to discover whether the supply of hormone from the thyroid gland is increased, whether its utilization by the tissues is increased or diminished, or whether for some reason its combination with serum protein is unusually indissoluble. Other explanations of the phenomena can be conceived, but these serve to indicate the nature of the problem. Some indirect evidence may be secured by analysis of the behavior during pregnancy of patients with excessive or deficient thyroid activity. The present paper, therefore, deals with the course of SPI in pregnancy of 16 patients with disorders of function of the thyroid gland.

## MATERIAL AND METHODS

The 16 patients were all under the care of the Metabolism Clinic and staff. The majority were delivered on the Obstetrical Service of Yale University School of Medicine in the New Haven Hospital. A small number were delivered elsewhere. The procedures and methods have been already described (3).

Of the 16 patients nine were definitely hyperthyroid. Of these, one had two, and two had three pregnancies. One patient was definitely, a second probably, and three others questionably, hypothyroid. One patient when first seen in pregnancy had indubitable hyperthyroidism, but after three pregnancies became hypothyroid. The last of the 16 had questionable hyperthyroidism. In all cases the treatment was altogether medical, consisting of the administration of dried thyroid, Lugol's solution, and either thiourea or propylthiouracil. With few exceptions, which are noted in the protocols, patients received 14 or 15 drops of Lugol's solution daily while they were taking thio drugs.

## PROTOCOLS OF CASES

Only features relevant to the subject of this paper are included in the following protocols.

B82863, born in 1917, had had a thyroidectomy for toxic goiter in 1939 and two operations for recurrences in 1946; one uncomplicated pregnancy in 1943, miscarriages in 1944 and 1946. When first seen, June 26, 1946, she had frank signs of hyperthyroidism with a diffuse goiter, slight exophthalmos, blood pressure 160/90, basal metabolism + 26 per cent, and SPI 12.5 $\gamma$ per cent. The record of SPI and antithyroid medication, beginning at this point, is illustrated in Figure 1. The course for 66 weeks preceding pregnancy is represented in broken lines to show the fluctuant character of the disease and SPI. During this period she was treated intermittently with Lugol's solution alone. When SPI was below 8.0 $\gamma$ per cent she was free from signs and symptoms; when it rose above 8.0 $\gamma$ per cent, hyperthyroidism manifested itself. The SPI of 8.8 $\gamma$ per cent just before pregnancy 4 was accompanied by symptoms and apparently marked an exacerbation of her disease, since thio drugs were required thereafter. Throughout the pregnancy and the early postpartum period she seemed to be in a euthyroid state; but 16 weeks after delivery, with SPI 10.3 γ per cent, there were signs of overactivity of the thyroid. Nevertheless, medication was stopped. She was evidently in a relapse 11 weeks before pregnancy 5 with SPI 18.4 γ per cent. This responded rapidly to propylthiouracil, the dose of which had to be reduced in the course of the pregnancy. Symptoms of hyperthyroidism did not recur until after 26 weeks and following interruption of the drug for two weeks SPI reached 13.2 γ per cent. Symptoms ceased again shortly after the SPI of 10.3 γ per cent at the end of 31 weeks.

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[1] This investigation was supported (in part) by a research grant from the U. S. Public Health Service, and by a grant from the Fluid Research Fund of the Yale University School of Medicine.

The hyperthyroidism in this case evidently progressed in severity during the period before pregnancy 4. The outburst of hyperthyroidism before pregnancy 5 can be attributed to inadvised discontinuation of therapy. The doses of thio drugs used in the two pregnancies are not so different as the figures would indicate because of the greater effectiveness of a given weight of thiourea. The initial dose was probably greater in pregnancy 5 while the later dose was greater in pregnancy 4 until the last period of 5 when the dose of propylthiouracil was increased to 100 mg. This may account for the differences in the two pregnancies. On the whole, however, the general similarity of the course of SPI is more striking. It fell rapidly in the early part of both pregnancies, despite diminishing doses of thio drugs, until it reached values lower than those usually encountered in pregnancy, rising again in the latter half of pregnancy. The sequences are complicated by the fact that the patient was in a hyperthyroid state just before both pregnancies. Initial treatment was obviously overvigorous at first and, in pregnancy 5, too light later. The complete withdrawal of propylthiouracil in this pregnancy was an error of clinical judgment. The rises of SPI as pregnancy advanced may have been due in part to the influence of pregnancy itself. In both pregnancies the patient remained free from symptoms when SPI lay between 8 and 10 γ per cent, the range for normal pregnancy, although symptoms of hyperthyroidism had appeared with SPI in the region of 8 γ per cent when she was not pregnant. This tolerance persisted for an interval after delivery in both instances, while SPI remained elevated. Subsequently, after pregnancy 4, hyperthyroidism appeared, with a further rise of SPI.

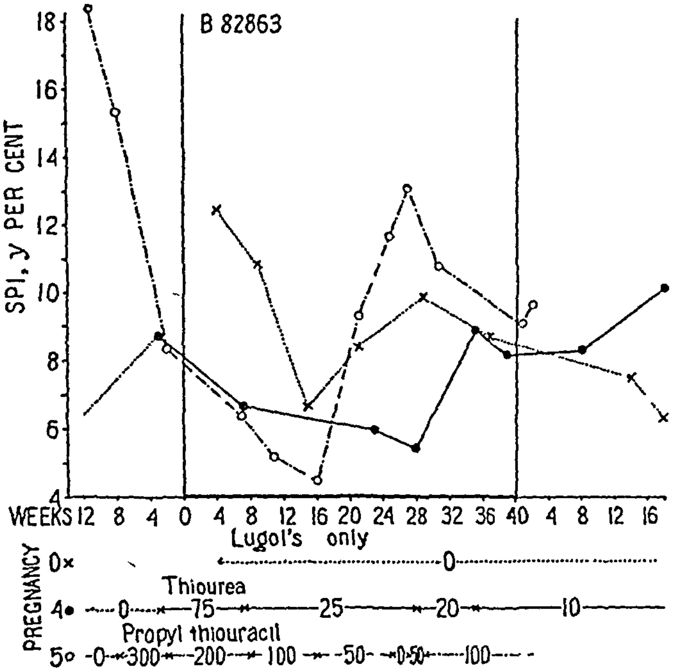

Fig. 1. The Course of SPI of a Hyperthyroid Patient Before and During Two Pregnancies

The period of pregnancy is defined by the two vertical lines. It is arbitrarily set at 40 weeks after the last menstrual period, 0 weeks. Observations are also dated from this period. The interval between the last observation in pregnancy and delivery is not, therefore, precise, since delivery did not always occur after exactly 40 weeks. Periods before and after pregnancy are indicated to the left and right of the pregnant area, respectively. Therapy is indicated by the figures below the chart, the lines and arrowheads indicating the duration of each dose.

In this particular case the broken line with solid dots does not represent a pregnancy, but a continuous period of 72 weeks preceding her fourth pregnancy during which the patient received only Lugol's solution (see protocol).

B50050, born in 1922, had normal deliveries in 1941 and 1942, miscarriages in 1941, 1942 and about July of 1945. Symptoms of hyperthyroidism began early in 1945. When first seen in March, 1946, she had diffuse enlargement of the thyroid, slight exophthalmos, frank hyperthyroidism, basal metabolism +68 per cent and SPI 24.2 γ per cent. The course of SPI and therapy, beginning with this point, which just preceded pregnancy, is illustrated in Figure 2. After six weeks, with SPI down to 9.3 γ per cent, symptoms and signs of hyperthyroidism had subsided. In neither pregnancy did symptoms or signs recur until the sharp peaks of SPI at 10 and 11 weeks postpartum, respectively.

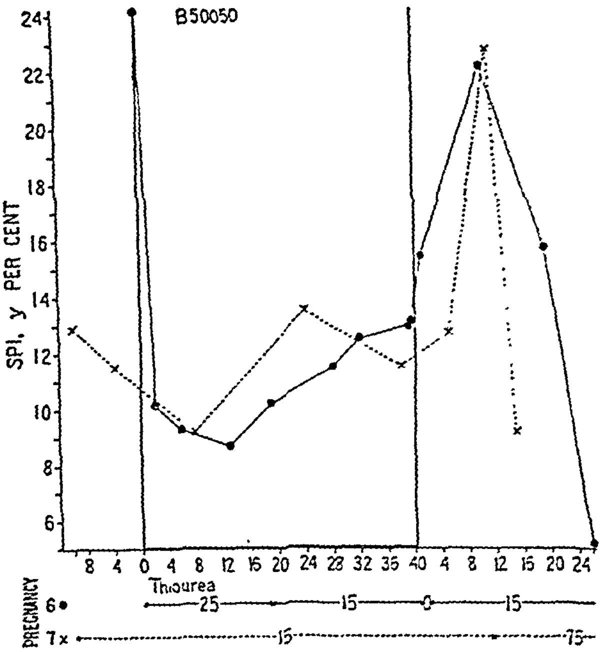

FIG. 2. THE COURSE OF SPI OF A HYPERTHYROID PATIENT IN TWO SUCCESSIVE PREGNANCIES

The general significance of the symbols is described in the legend to Figure 1. Only a part of the interval between pregnancies is illustrated.

The similarity of the course of SPI in these two pregnancies seems too close to be ascribed to coincidence. The slight early decline and the late rise resemble the reactions of the preceding case (B82863), but the picture is not distorted by changes of therapy or the severity of the thyroid disorder, which appears to have remained relatively stable. The impression is derived that in pregnancy hyperthyroidism can be more easily controlled. In this sense hyperthyroidism is not synonymous with SPI which appears to follow a course similar to that characteristic of pregnancy in euthyroid subjects, but perhaps at a higher level. The outbursts of hyperthyroidism after each delivery suggest a discharge of activity after the release of the inhibitory effect of pregnancy. The figures in the later part of pregnancy are so high as to indicate that this condition conferred a degree of tolerance for thyroid hormone (or SPI) greater than the similar tolerance of euthyroid pregnant subjects. In this case, however, this can not be definitely substantiated. Just before pregnancy 7, while the patient appeared to be in a euthyroid state, values of 12.9 and 11.5 γ per cent were observed.

B35786, born in 1922, had a normal pregnancy in 1943. In 1940 and in 1944 she was given small doses of thyroid because of increasing weight and lethargy. Symptoms of hyperthyroidism appeared early in February, 1946. She was seen March 7 of that year, four weeks pregnant, with moderate enlargement of the thyroid, typical manifestations of hyperthyroidism, basal metabolism, +56 per cent and SPI 23.1 γ per cent. Her course, beginning with this point, is illustrated in Figure 3. There was a temporary sharp fall of SPI, but symptoms were not allayed and basal metabolism remained elevated until about the 24th week. She was again distinctly hyperthyroid 11 weeks after delivery when SPI had shot up to 23.8 γ per cent and continued to have symptoms, except during a short remission when SPI fell to 6.2, until pregnancy 3. In the first part of this pregnancy, which terminated in a spontaneous miscarriage after 23 weeks, hyperthyroidism was not allayed and SPI rose, probably because the patient was unable by reason of nausea to take her medication. Three days after the miscarriage (the interval in the figure is not real, but represents only transition from pregnancy to the postpartum state) basal metabolism was +3 per cent and SPI had fallen to 11.1 γ per cent. Thirty-five weeks after the miscarriage she was again thyrotoxic and came into pregnancy 4 in this state. She became euthyroid, however, shortly after pregnancy began and remained in this state as long as she was followed.

In this case again, after the initial hyperthyroidism was controlled, SPI declined steadily in both pregnancy 2 and pregnancy 4, remaining, however, always above nonpregnant euthyroid con-

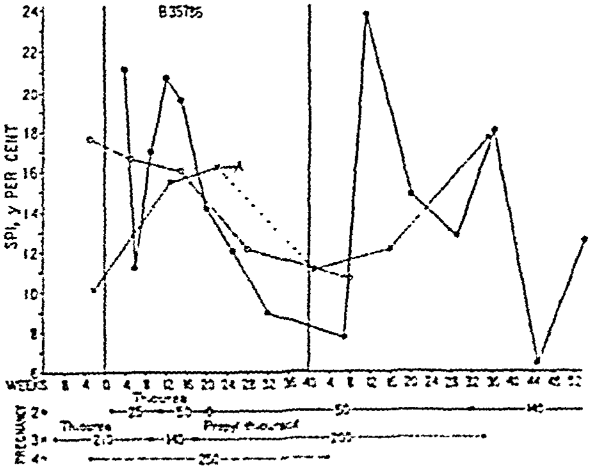

FIG. 3. THE COURSE OF SPI OF A HYPERTHYROID PATIENT IN THREE SUCCESSIVE PREGNANCIES

The general significance of the symbols is described in the legend to Figure 1. Pregnancy 3 ended in abortion at the point A. The interval between this and the vertical line marking the end of pregnancy does not indicate actual elapsed time. The next observation was made one week after the abortion.

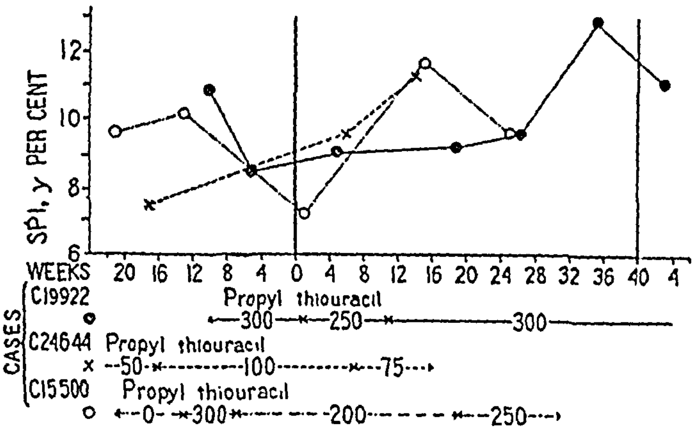

FIG. 4. THE COURSE OF SPI OF THREE HYPERTHYROID PATIENTS DURING PREGNANCY

The general significance of the symbols is described in the legend to Figure 1.

centrations, suggesting that the requirement for thio drug diminished. Pregnancy 3 can not be evaluated. The course of SPI might suggest that the hyperthyroidism was exacerbated, the rise of SPI can probably be attributed to her failure to take her medication. After pregnancy 2 she had an outburst of hyperthyroidism associated with a sharp rise of SPI quite similar in degree and time to those of the preceding case. This only partly subsided spontaneously. Although no sharp spike was noted after pregnancy 3, SPI rose after pregnancy. At 36 and 35 weeks, respectively, the values for SPI after pregnancies 2 and 3 were almost identical. The patient appeared to tolerate while pregnant values of SPI higher than those tolerated even during pregnancy by normal persons.

C19922, born in 1913, when first seen on January 4, 1949, had four children, the oldest 11. Symptoms of hyperthyroidism, which began four months earlier, were characteristic, including a slight goiter and mild exophthalmos, basal metabolism +34 per cent and SPI 10.9 γ per cent. Her course beginning at this point is illustrated in Figure 4. Symptoms were rapidly allayed, but recurred when the dose of thiouracil was reduced during the early part of pregnancy.

C24644, born in 1914, developed symptoms of hyperthyroidism shortly after a normal delivery in 1946. When first seen, April 16, 1948, after she had taken 150 mg. of propylthiouracil daily for three weeks, she was still definitely hyperthyroid, with a diffusely enlarged thyroid and SPI 7.1 γ per cent. On Lugol's solution and 50 to 125 mg. of propylthiouracil daily, symptoms and signs were kept in abeyance and SPI within normal limits until the onset of her pregnancy, January 17, 1950. The record in Figure 4 begins 17 weeks before this. Although, after the onset of pregnancy, without change of medication, SPI rose, she remained euthyroid.

C15500, born in 1920, had a normal pregnancy in 1946. In 1942 she developed hyperthyroidism, which recurred after a thyroidectomy in 1944. When first seen, August 30, 1948, she had mild symptoms of hyperthyroidism and SPI 9.6 γ per cent. The course beginning with this point is illustrated in Figure 4. Symptoms subsided rapidly after propylthiouracil was begun. Thereafter, with the exception of a short interval at about 16 weeks when SPI was 11.7 γ per cent, she seemed euthyroid.

SPI in the three cases illustrated in Figure 4 remained at more moderate concentrations than it did in the three preceding cases. The observations were, however, limited. In all, insofar as they were studied, SPI tended to rise as pregnancy advanced. With these rises C19922 and C15500 developed or retained some symptoms and signs suggestive of continued overactivity of the thyroid. These are the only patients in the series who had such manifestations while SPI was below 10 γ per cent. In contrast, SPI of C24644 rose during pregnancy, without evidences of hyperthyroidism, to concentrations far above those that before pregnancy had been associated with symptoms.

C38870, born in 1919, had an uncomplicated pregnancy in 1942. At that time she was given 0.03 gm. of thyroid daily. In November, 1944, she developed symptoms of hyperthyroidism, accompanied by urticaria, angioneurotic edema and bronchial asthma. When seen in this condition September 9, 1945, her basal metabolism was +36 per cent, SPI 10.4 γ per cent. The record of her course in Figure 5 begins eight weeks before the onset of pregnancy, which began June 15, 1949. In spite of the steady rise of SPI during pregnancy, she had no evidences of hyperthyroidism until eight weeks after delivery when SPI was 16.1 γ per cent.

B87633, born in 1909, had normal pregnancies in 1940 and 1943. After a thyroidectomy in 1940, hyperthyroidism recurred in 1945. When first seen, May 29, 1946, 23 weeks after the beginning of pregnancy, she had a diffuse goiter, basal metabolism +24 per cent and SPI 13.3 γ per cent. Her course beginning with this point is illustrated in Figure 5. She had a remission of symptoms shortly after medication began and just after delivery, corresponding to the low SPI's, but had a slight recurrence of thyrotoxicosis just before delivery and a more obvious one eight weeks after delivery.

ST, born in 1918, developed hyperthyroidism in 1944. When first seen, May 8, 1947, taking probably 150 mg. of propylthiouracil and 15 drops of Lugol's solution daily, her thyroid was moderately enlarged, but she had no signs of hyperthyroidism. SPI proved to be 1.9 γ per cent. The course, beginning with this point, nine weeks before pregnancy, is illustrated in Figure 5.

With reduction of the dose of propylthiouracil SPI rose quite slowly at first, but between 11 and 16 weeks jumped from 4.9 to 9.2 $\gamma$ per cent. Just before delivery, the dose of propylthiouracil was increased because of transitory equivocal symptoms of hyperthyroidism: palpitation and sweating, but no tachycardia, basal *metabolism +5 per cent and SPI 8.3 $\gamma$ per cent.* With the SPI 20.6 $\gamma$ *per cent 12 weeks after delivery* she was frankly hyperthyroid with pulse rate 102 and basal metabolism +55 per cent. The infant at birth had a large goiter. This will be discussed in another communication.

The three cases in Figure 5 suggest again that the requirement for thio drugs, estimated by clinical criteria, diminishes, although SPI may rise, as pregnancy advances. They also suggest that SPI may rise higher in pregnancy and for a short interval after pregnancy than in the nonpregnant state, without evidence of hyperthyroidism. They also suggest that the hyperthyroid patient can tolerate higher values than the euthyroid subject can during pregnancy. In case C38870 reduction of thiourea from 75 to 25 mg. may have been unwarranted. Nevertheless, it resulted in *no precipitate rise of SPI*, but a gradual, symptomless ascent from 8.4 to 12.1 $\gamma$ per cent. Case *B87633, on 15 mg. of thiourea daily,* was not unquestionably euthyroid when SPI fluctuated between 13.3 and 10.2 $\gamma$ per cent; but, after the first observation, signs of hyperactivity were minimal. After delivery all three patients developed hyperthyroidism, C38870 and B87633 without change of therapy, ST despite an increased dose of thiourea. In B87633 these symptoms were not associated with a greatly elevated SPI, but in the other two patients they were. ST exhibited a sharp peak similar to those observed in B50050 (Figure 2) and B35786 (Figure 3) at approxi*mately the same interval after delivery.* ST, when first seen, was evidently approaching myxedema. *Reduction of the dose of propylthiouracil* to 100 mg. raised SPI only to 4.9 $\gamma$ per cent in 15 weeks. At the end of that time, however, it rose abruptly without change of therapy, behaving like that of a normal pregnant woman.

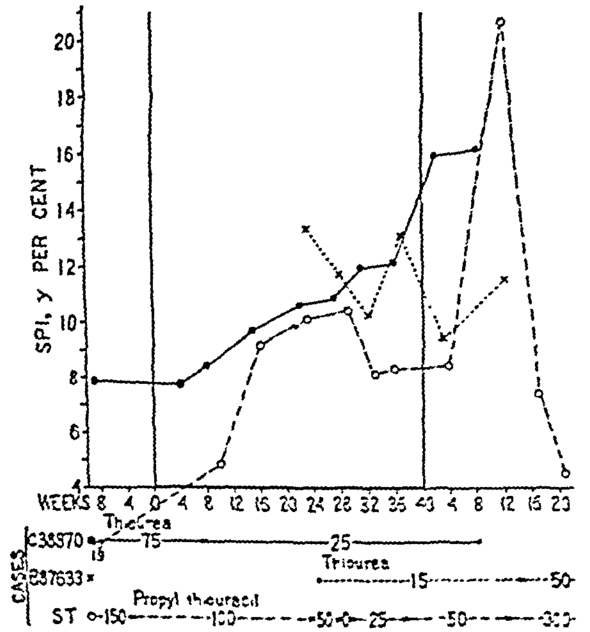

FIG. 5. THE COURSE OF SPI OF THREE HYPERTHYROID PATIENTS DURING PREGNANCY

The general significance of the symbols is described in the legend to Figure 1.

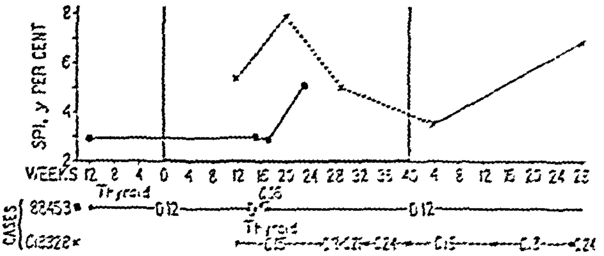

FIG. 6. THE COURSE OF SPI OF TWO HYPOTHYROID PATIENTS DURING PREGNANCY

The general significance of the symbols is described in the legend to Figure 1.

*88453, born in 1919, had a pregnancy in 1938.* In 1939 she noted increasing weight, puffiness of the eyelids and legs, and increasing fatigue. When first seen on September 16, 1942, she presented classical signs of myxedema. SPI was 0.4 $\gamma$ per cent, basal metabolism −33 per cent and serum cholesterol 249 mg. per cent. On 0.12 gm. of thyroid daily her condition improved rapidly, SPI varying from 3.0 to 5.4 $\gamma$ per cent, basal metabolism from −14 to +7 per cent, in the next 15 months. She became pregnant January 1, 1944. SPI 11 weeks before this was 2.9 $\gamma$ per cent. *Her course, beginning with this point, is illustrated in Figure 6.* Subsequent SPI's in 1949, while she was still taking 0.12 gm. of thyroid, were 3.0 and 3.4 $\gamma$ per cent. Clinically she remained euthyroid throughout pregnancy on the same dose of thyroid that she took when not pregnant.

C18328, born in 1922, in 1944 noted increasing weight, somnolence, lassitude, dry nails and brittle hair. In 1945, 0.06 gm. of thyroid daily, given because her metabolism was −26 per cent, restored her to a normal state. When she stopped the thyroid that summer symptoms recurred, basal metabolism falling to −28 per cent. She was given 0.12 gm. of thyroid, which was increased in March, 1946, to 0.15 gm. daily. When first seen on November 15, 1948, after the 12th week of pregnancy, she appeared normal in all respects, with SPI 5.4 γ per cent and basal metabolism − 11 per cent. The record in Figure 6 begins with this point.

Of the two cases illustrated in Figure 6, one, 88453, had frank myxedema; the second probably had hypothyroidism. Unfortunately, the observations on both, in and about pregnancy, are scanty. Case 88453 had extremely low SPI's at 15 and 17 weeks, 3.0 and 3.4 γ per cent, respectively. This would seem to indicate that such values are not incompatible with completion of a normal pregnancy. She had, however, omitted thyroid for nine days before the first of these and had taken an inadequate dose in the interval of three weeks before the second. The two low values may, therefore, represent a transitory drop. With resumption of a proper maintenance dose, SPI at the next observation was 5.1 γ per cent and may have risen farther as pregnancy advanced. Case C18328, when first seen in pregnancy, had an SPI of 5.4 γ per cent that rose, at the end of eight weeks, without change of therapy, to 7.9 γ per cent. In the later part of pregnancy it had fallen to 5.0 γ per cent, although the dose of thyroid had been increased. Unfortunately, no further observations were made until after delivery when, on the original dose of thyroid, SPI was 3.5 γ per cent. This patient was never seen when she was not taking thyroid. The story of her initial symptoms and her reactions to administration and withdrawal of thyroid seem characteristic of hypothyroidism. Nevertheless, SPI was never subnormal. Furthermore, it rose spontaneously under the stimulus of pregnancy, as it does in normal persons, without change of therapy, and fell after delivery. Finally, she was able after delivery to tolerate 0.24 gm. of thyroid without clinical evidence of hyperthyroidism, which is unusual for a hypothyroid subject. The effect of this dose on SPI was, unfortunately, not ascertained. Even 88453, with indubitable hypothyroidism, seemed to be able to raise SPI under the stimulus of pregnancy.

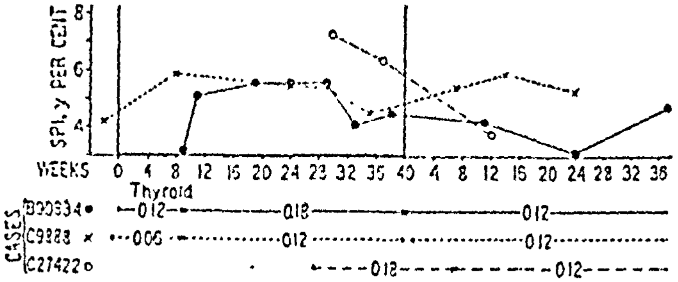

FIG. 7. THE COURSE OF SPI OF THREE QUESTIONABLY HYPOTHYROID PATIENTS DURING PREGNANCY

The general significance of the symbols is described in the legend to Figure 1.

B90834, born in 1924, in 1940 was given up to 0.30 gm. of thyroid daily for obesity. This dose was later reduced to 0.12 gm., and in 1945 discontinued entirely for eight months, without noticeable effects. Thyroid was again resumed in a dose of 0.09 gm. because the basal metabolism was − 16 per cent, with the idea that it might promote conception. In 1947 she had a normal delivery after an uneventful pregnancy. November 3 of the same year her basal metabolism was − 17 per cent. December 23 thyroid was discontinued. January 13, 1948, she had a prolonged menstrual period at the normal interval. Because a spontaneous abortion was suspected, she was ordered to resume 0.12 gm. of thyroid daily, despite the absence of evidences of hypothyroidism. On several occasions after this, because of irregular bleeding, pregnancy was suspected, but the Ascheim-Zondek test remained consistently negative. March 3 the dose of thyroid was increased to 0.18 gm., but within a month was again reduced to 0.12 gm. and finally, on October 18, was stopped entirely. The interruption coincided with her last menstrual period. December 20, when the first SPI was 3.2 γ per cent, she had been pregnant for nine weeks and had been taking 0.12 gm. of thyroid for five weeks. The course, beginning with this point, is illustrated in Figure 7.

C9888 was born in 1927. In 1940, because she lacked vigor, was somewhat somnolent and had oily skin, a basal metabolism was taken and found to be low. In 1942 her condition appeared to improve when she took 0.06 gm. of thyroid daily. In January, 1947, just before marriage, she discontinued medication with no other effect than slight retardation. January 22, 1948, she became pregnant. Her SPI two weeks earlier was 4.2 γ per cent, basal metabolism − 16 per cent. Her course, beginning with this point, is illustrated in Figure 7.

C27422, born in 1920, in 1947, because of lassitude, infertility and a basal metabolism of − 23 per cent, was given 0.12 to 0.18 gm. of thyroid daily. She noticed little change in her symptoms, but her basal metabolism rose to − 5 per cent, to fall again to − 17 per cent when she discontinued medication. She, therefore, resumed thyroid, taking from 0.03 to 0.12 gm. daily.

She became pregnant February 26, 1949. May 31, since she had no signs of thyroid disorder, the thyroid was discontinued. June 27, because her basal metabolism was − 19 per cent, she was given 0.09 gm. of thyroid daily, which was progressively increased until on September 8 it was 0.18 gm. September 26, at the end of 30 weeks of pregnancy, SPI was 7.3 γ per cent, pulse rate 72. The course, beginning with this point, is illustrated in Figure 7.

The three patients in Figure 7 at one time or another received thyroid for presumptive hypothyroidism, although it is doubtful whether this diagnosis was justified. SPI of B90834 and C9888, on moderate doses of thyroid, rose in the early months of pregnancy to concentrations of 5.6 to 5.9 γ per cent, slightly lower than those usual in pregnancy, falling in the later part of pregnancy without change of therapy. The course of C27422 can not be distinguished in any respect from that of a normal pregnant woman. The trio emphasized what was brought out in an earlier paper (3), the ability of the pregnant woman to dispose of thyroid hormone.

A16616, born in 1907, on June 24, 1942, when she was six weeks pregnant, though free from symptoms, was found to have a palpable thyroid gland, slight exophthalmos, a pulse rate of 92, basal metabolism +35 per cent and SPI 11.2 γ per cent. Her course, beginning at this point, is illustrated in Figure 8. She was followed altogether through three pregnancies and the intervals between them, receiving variable, sometimes intermittent, doses of Lugol's solution. Her course throughout resembled that of a normal woman. There were never clinical manifestations of hyperthyroidism and the pulse rate did not exceed 80.

C15718, born in 1926, in 1945 noted enlargement of her thyroid gland. In 1946 she had a normal pregnancy. When seen September 2, 1948, she had a moderate, diffuse enlargement of the thyroid, rather dry hair, a pulse rate of 68, basal metabolism −22 per cent, SPI 3.8 γ per cent and serum cholesterol 197 mg. per cent. Her course, beginning with this point, is illustrated in Figure 8. There was nothing unusual about her course until 14 weeks after delivery when she was found to be gaining weight rather rapidly, her pulse rate was only 52 and she complained of pains in the upper part of her chest and feeling bloated after meals. One month later her weight had increased further, her skin had become dry and her face and hands seemed puffy. Serum cholesterol proved to be 358 mg. per cent (unfortunately the tube containing the specimen for determination of SPI was broken). She was given at once 0.12 gm. of thyroid, which was increased to 0.18 gm. daily four weeks later, because her symptoms had not been entirely allayed. April 10, 1950, two months after the thyroid was begun, SPI was 6.3 γ per cent, serum cholesterol 135 mg. per cent, pulse rate 70, weight normal, and she felt and appeared euthyroid.

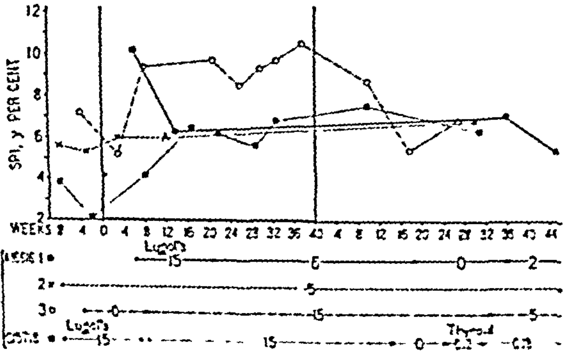

FIG. 8. THE COURSE OF SPI OF TWO QUESTIONABLY HYPERTHYROID PATIENTS DURING PREGNANCY

The general significance of the symbols is described in the legend to Figure 1. Case A16616 was followed through three pregnancies. The figures for therapy refer to drops of Lugol's solution except at the end of the course of Case C15718 when thyroid was substituted.

These two cases illustrated in Figure 8 are quite anomalous. There can be little doubt that A16616 had hyperthyroidism when she was first seen. Both SPI and evidences of hyperthyroidism *subsided under the influence of Lugol's solution* alone. Thereafter she ran a clinically almost euthyroid course in and out of pregnancy, with and without Lugol's solution in variable doses. On one occasion, after the third delivery, moderate signs of overactivity of the thyroid appeared with SPI 8.0 γ per cent, while she was receiving 5 drops of Lugol's solution daily. These subsided without change of therapy, SPI falling to 5.5 γ per cent, and subsequently to 3.4 γ per cent while she was taking no medication. The basal metabolism also was most capricious, usually above normal, with no apparent relation to SPI or clinical condition. This is a most abnormal pattern of behavior. Whether it indicated fluctuant thyroid activity it is impossible to say. Whatever its significance may have been, in pregnancy SPI followed the course expected in a euthyroid subject.

Case C15718 was given Lugol's solution in behalf of a goiter only. Shortly thereafter, just before pregnancy, SPI fell to 2.1 γ per cent, a concentration indicative of hypothyroidism. Nevertheless, it rose in pregnancy and remained elevated until 14 weeks after a normal delivery, when she suddenly developed hypothyroidism. It is possible that she was entering this state before pregnancy. In this case it must be inferred that the stimulus of pregnancy was able to elicit a response from the failing gland.

## DISCUSSION

Certain difficulties present themselves in the analysis of these data. Hyperthyroidism is inherently a variable disease, marked by remissions and exacerbations. In many instances there was no opportunity to observe the behavior of the patients for extended periods outside of pregnancy. Sometimes only fragments of the courses during pregnancy could be studied. Often it was not feasible to make observations at frequent or consistent intervals. The most desirable intervals for observations, indeed, were not known. It has been pointed out that SPI fluctuates greatly during normal pregnancy (3). It was impossible to follow any consistent course of therapy with justice to the patients; the treatment had to be regulated in accordance with the presumptive needs of each patient. In the earlier phases of the study the peculiar behavior of SPI during pregnancy was unknown. Attempts were, therefore, made to keep SPI within normal, nonpregnant limits. These were, however, somewhat tempered by fear for the consequence to the fetus should thyroid function be too much depressed. Efforts to strike a golden mean were not entirely successful. Recognition of the facts about SPI in pregnancy only complicated the problem. The last purely objective measure of thyroid activity was removed. Both basal metabolism and serum lipids are variously affected by pregnancy and are not, therefore, reliable indices of thyroid function in this state. Although both were measured at intervals, reliance had to be placed chiefly on clinical appraisal of the patients' conditions.

In spite of these difficulties the case material is large and varied enough to warrant some tentative deductions. In most instances SPI during pregnancy in patients with thyroid disorders, as in euthyroid subjects, was greater than 6.0 γ per cent. In some instances, usually as a result of treatment, it fell below 6.0 γ per cent temporarily or did not reach this concentration until pregnancy had advanced to 16 weeks. Occasionally it declined in the terminal weeks of pregnancy. In only two patients, B90834 and C9888 (Figure 7), did it remain persistently below 6.0 γ per cent, and in these two it was above 5.0 γ per cent until 33 weeks. In general it tended to follow the course observed in normal pregnancy, rising in the course of the first 16 weeks, usually remaining elevated until delivery. The time of the initial rise varied from case to case and was frequently modified by treatment; but in cases with more than one pregnancy there was a definite tendency for the same pattern of behavior to recur despite variable treatment.

In patients with active hyperthyroidism the SPI curves tended to lie at higher concentrations than the curves of euthyroid pregnant patients. Just as the latter tolerate in pregnancy concentrations of SPI that in nonpregnant women would be indicative of hyperthyroidism, so the hyperthyroid patient appeared to tolerate still higher values without symptoms. It was not possible to demonstrate this in all the subjects because some, for example B50050, when not pregnant, on occasions had unusually high SPI without hyperthyroidism. In the hyperthyroid subjects, however, this combination occurred with far greater frequency. Only two patients, C19922 and C24644, had symptoms suggestive of persistent hyperthyroidism during pregnancy with SPI under 10 γ per cent, and in these two the signs were equivocal. This does not imply that the tolerance of the hyperthyroid to high SPI is actually greater than that of the euthyroid subject because the SPI of the latter is not easily raised to the concentrations encountered in the hyperthyroid subjects. It merely indicates that in pregnancy higher SPI can be tolerated even if this arises from overactivity of the thyroid gland. This is circumstantial evidence that the increment in pregnancy is composed of thyroid hormone. The ability of the pregnant woman to dispose of exogenous hormone was demonstrated in the preceding paper. It is also evident in the three patients whose courses are shown in Figure 7.

In those patients in which antithyroid treatment was kept unchanged or was reduced during pregnancy, the course of SPI and symptoms, especially the latter, suggest that during pregnancy the requirement for antithyroid therapy diminishes. This might be interpreted as an indication that the tolerance for thyroid hormone is increased, a view that is in keeping with the traditional concept that the activity of the thyroid gland increases during pregnancy.

A further indication that the tolerance for thyroid is increased and the need for therapy diminished during pregnancy is found in the sudden rises of SPI associated with outbursts of hyperthyroidism encountered after delivery in a large proportion of the cases, especially the flagrant outbursts associated with high peaks of SPI. These were encountered on four occasions in nine patients with definite hyperthyroidism. The three patients in whom they occurred were all followed for long periods without other similar incidents. All the peaks were noted within the narrow interval of 10 to 12 weeks after delivery . Since they appeared to be wholly or partly self-terminative and of short duration, they might have been discovered more often if observations postpartum had been made with greater frequency. Lesser rises of SPI associated with hyperthyroidism were noted after delivery in several other instances. These rises associated with hyperthyroidism differ from the persistently high values immediately after delivery, which are symptomless. SPI does not always fall immediately after delivery in hyperthyroid patients any more than it does in normal subjects (3); but may stay elevated for some time. These elevations, like those after normal pregnancy, are not associated with signs of hyperthyroidism. The tolerance to SPI is not coterminal with pregnancy but outlasts it by an appreciable interval. Both tolerance and interval may be greater in the thyroid patient. But, if the rise persists beyond a certain time, symptoms appear. The SPI of C38870 (Figure 5), for instance, rose to 15.9 $\gamma$ per cent 11 days after delivery without evidences of hyperthyroidism, but six weeks later with the same concentration of SPI she had distinct symptoms.

The tendency for SPI to rise during pregnancy was evident even in the patients with hypothyroidism. This might suggest that the high SPI signified not increased secretion and utilization, but some block to the utilization of thyroid hormone. This interpretation appears unlikely, first of all because of the tolerance for exogenous thyroid evinced by euthyroid pregnant women (3). The lesser need for antithyroid therapy would also argue against such an hypothesis, unless it be also presumed that the secretory activity of the thyroid is decreased during pregnancy, which would make it difficult to explain the high values of SPI. The rises after delivery would be equally unaccountable on this basis. It would also be necessary on the diminished utilization theory to hypothesize that the tissues required less thyroid during pregnancy, else the hypothyroid patients should have sunk into myxedema as SPI rose. The SPI of case B82863 (Figure 1) shot up with the appearance of active hyperthyroidism after propylthiouracil had been discontinued for only two weeks, descending with equal speed when the previous dose of drug had been resumed. This rapid reaction indicates that secretion, utilization and sensitivity are all active. It has been remarked that in the authentic hypothyroid, 88453 (Figure 6), the low SPI found at 15 weeks followed abstention from medication for only one week. If it be assumed that it had previously been as high as it later was on the original dose of 0.12 gm., this would suggest an unusually rapid deterioration. C18328, in the same figure, appeared to utilize increasing amounts of thyroid in the later part of pregnancy. It is probable that clinical hypothyroidism in many cases is not a total, but a partial, deficiency. In some cases, indeed, it may represent only the absence of the normal stimulus to thyroid activity. It is conceivable that the stimulus of pregnancy may break down this inhibition or arouse the residual thyroid tissue to activity. In this connection the response of C15718 (Figure 8) is most suggestive.

In the preceding paper (3) it was pointed out that the SPI's of women receiving exogenous thyroid could remain low without compromising pregnancy. In the present series persistently low SPI was encountered only in patients who were receiving exogenous thyroid.

The argument thus far has presupposed that the increment of SPI in pregnancy is of the same composition as the preexisting SPI, chiefly thyroid hormone. This presumption is open to challenge. Danowski and associates (5) found that extraction of the serum with butanol by the method of Taurog and Chaikoff (6) yielded values in pregnancy that did not differ from SPI more than the values by the two methods differed in the sera of normal individuals. Similar results have been obtained by us in a number of sera (7). In this respect, at least the increment in pregnancy behaves like thyroxine. In addition, the reaction of euthyroid pregnant women to thyroid and the behavior of the patients with thyroid disorders presented in this paper are more easily explained on the hypothesis that the increment of SPI does consist of thyroid hormone.

## SUMMARY AND CONCLUSIONS

The clinical course and the SPI during pregnancies of 16 patients with disorders of thyroid function have been analyzed. Treatment was limited to the use of thiourea, propylthiouracil, Lugol's solution and dried thyroid. The hyperthyroid patients became and remained euthyroid with concentrations of SPI characteristic of normal pregnancy, distinctly above those of normal nonpregnant women. There is some evidence that they were able to tolerate in pregnancy concentrations greater than 10 γ per cent, the highest value observed in euthyroid pregnant subjects. The data suggest that the requirements for antithyroid drugs may diminish during pregnancy and increase after delivery.

The implications of these phenomena with respect to the nature and significance of the high SPI of pregnancy are discussed.

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crepitation on motion was noted. There was thickening of the periarticular tissues.

Shortly after admission, she had a febrile episode associated with a urinary tract infection and lapsed into complete unresponsiveness with further drop in blood pressure. Treatment for Addisonian crisis was instituted and resulted in a satisfactory response. An unsuccessful attempt to stabilize the blood pressure, electrolyte, and endocrine metabolism on desoxycorticosterone and thyroid extract was made. Eventual maintenance medications were: *1*) Lipoadrenal Cortex (Upjohn), 2 ml. intramuscularly daily. *2*) Testosterone propionate, 20 mg. intramuscularly daily. *3*) Sodium chloride, 6 gm. daily.

Laboratory examination at the time of admission failed to demonstrate the presence of urinary gonadotropins, and 17-ketosteroid excretion was 0.84 mg./24 hours. Serum sodium was 290 mg./100 ml., potassium 29.3 mg./100 ml., and plasma chlorides 525 mg./100 ml. Blood urea nitrogen was 17 mg./100 ml. and the creatinine 1.0 mg./100 ml. X-rays revealed the lung fields to be clear. The cardiothoracic ratio was 0.38. X-rays of the abdomen demonstrated no calcification in the adrenal areas. Skull x-rays were normal. Roentgenograms of the hands demonstrated atrophic changes. A basal metabolic rate two weeks after admission was −16%. A plasma protein bound iodine in June, 1950, was 2.6 gamma/100 ml. A test for adrenal cortical insufficiency with 25 mg. ACTH as described by Thorn, Forsham, Prunty, and Hills (9) failed to demonstrate a significant drop in circulating eosinophils or change in urinary uric acid excretion. An intravenous glucose tolerance test produced a febrile reaction and drop in blood pressure which necessitated discontinuance of the test and use of supplementary adrenal cortical extract and intravenous saline.

## METHODS

During the study the carbohydrate, fat, protein, and sodium in the diet were calculated since it was impossible to keep the diet constant. Blood pressure and weight were recorded daily at 7:00 a.m. Because of the mental status of the patient 24 hour urine and stool specimens were collected with some difficulty. Toluene was used as preservative in the urine specimens except on days when 17-ketosteroid and uric acid determinations were made. Eosinophil counts and leukocyte counts were done by the method of Randolph (10); erythrocyte sedimentation rates by the Westergren method; serum so-

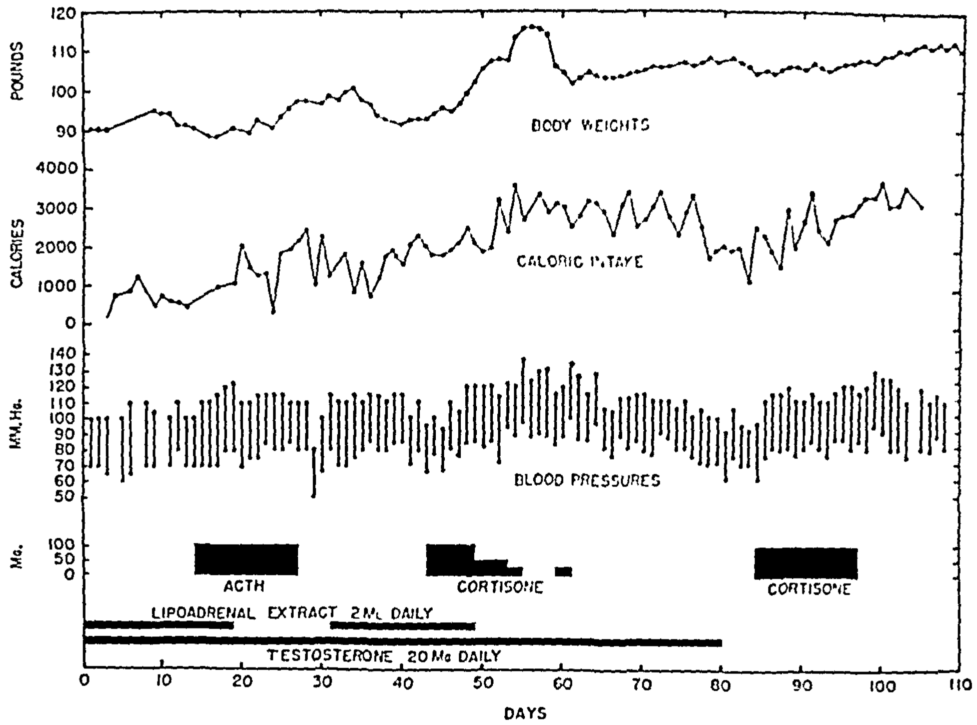

FIG. 2

dium by the method of Hoffman and Osgood (11); serum potassium according to Breh and Goebler (12); urine and blood uric acid by the method of Folin (13); 17-ketosteroids by the method of Robbie and Gibson (14). The patient was studied daily by two independent observers, and notations were made of changes in mental status, joint mobility, and general appearance. ACTH (Armour Standard LA-1-A Control J9801) freshly dissolved in saline was administered intramuscularly, 25 mg. every six hours. Cortisone acetate (Merck) was given every six hours intramuscularly (Figures 2, 3, 4).

*Regimen* (Figure 2): Function of the pituitary was thought to be so slight that to discontinue all medication at the start of ACTH administration was inadvisable. Lipoadrenal cortex was omitted after four days of ACTH, but testosterone was continued. Lipoadrenal cortex was again given at the termination of the course of ACTH when pneumonitis with fever and hypotension intervened. During the first period on cortisone, lipoadrenal cortex was discontinued when a sharp rise in weight and elevation of blood pressure occurred. Shortly thereafter cortisone was reduced and, later, discontinued when edema appeared. Added sodium chloride was also discontinued. Testosterone was discontinued before the second course of cortisone. At the completion of the second course of cortisone, no medications were given for 21 days. Fall in blood pressure necessitated resumption of therapy with lipoadrenal cortex.

## RESULTS

*Clinical observations:* Before the beginning of this study, the patient was lethargic, passive and intermittently hallucinated. During the first six days of ACTH administration, hallucination and agitation become extreme, requiring restraint and on one occasion tube feeding. The psychotic behavior became less pronounced when ACTH was continued. While on cortisone the patient became cooperative, and had long periods of lucid behavior. During administration of cortisone the skin remained dry and soft. The subcutaneous tissue of the face took on a loose, spongy consistency much like that seen in myxedema. A luxuriant growth of axillary and pubic hair appeared during cortisone therapy. This was accompanied by extreme modesty, which heretofore had been lacking completely. The activity of the rheumatoid arthritis was such that no dramatic change was observed. During the course of ACTH there was slight objective increase in range of motion of the knees, ankles and left elbow. After four days on ACTH the patient walked spontaneously for the first time during the entire period of hospitalization. The agitation and hallucination at this period, however, probably accounted for the apparent absence of pain on walking. In the interval between ACTH and cortisone administration there was a definite exacerbation of joint pain, swelling and local heat. Cortisone brought about definite objective and subjective improvement in the rheumatoid state (Figure 1). This occurred gradually during a seven day period. During the 22 day period between the courses of cortisone there was no exacerbation of joint signs or symptoms. Twenty-one days after completion of the second course of cortisone, both knees but especially the right, became painful, warm, and swollen. An accompanying systemic febrile response occurred.

A gain in body weight of nine pounds was noted after 10 days of ACTH administration (Figure 2). No edema was evident. This gain could not be followed accurately because pneumonitis necessitated the use of supplementary adrenal cortical extract three days after completion of ACTH administration. By the sixth day of the first course of cortisone, a weight gain of 10 pounds had occurred. Edema appeared and remained despite discontinuation of lipoadrenal cortex and sodium chloride. Cortisone was discontinued on the 12th day after her weight had risen from 93 to 115 pounds. It was believed that the appearance of edema was a manifestation of sodium retaining effect of cortisone (3), added to the effect of testosterone (15). After edema had disappeared her weight remained 10 pounds above the control level and represented an increase in body tissue. This gain was maintained in the 23 day interval between courses of cortisone. During the second period of cortisone her weight increased but 3 pounds. However, an additional gain of 5 pounds was noted in the 12 day interval subsequent to its administration. During the second period, when cortisone was the only drug being administered, no edema appeared.

Blood pressure was maintained slightly above control levels by ACTH after discontinuation of

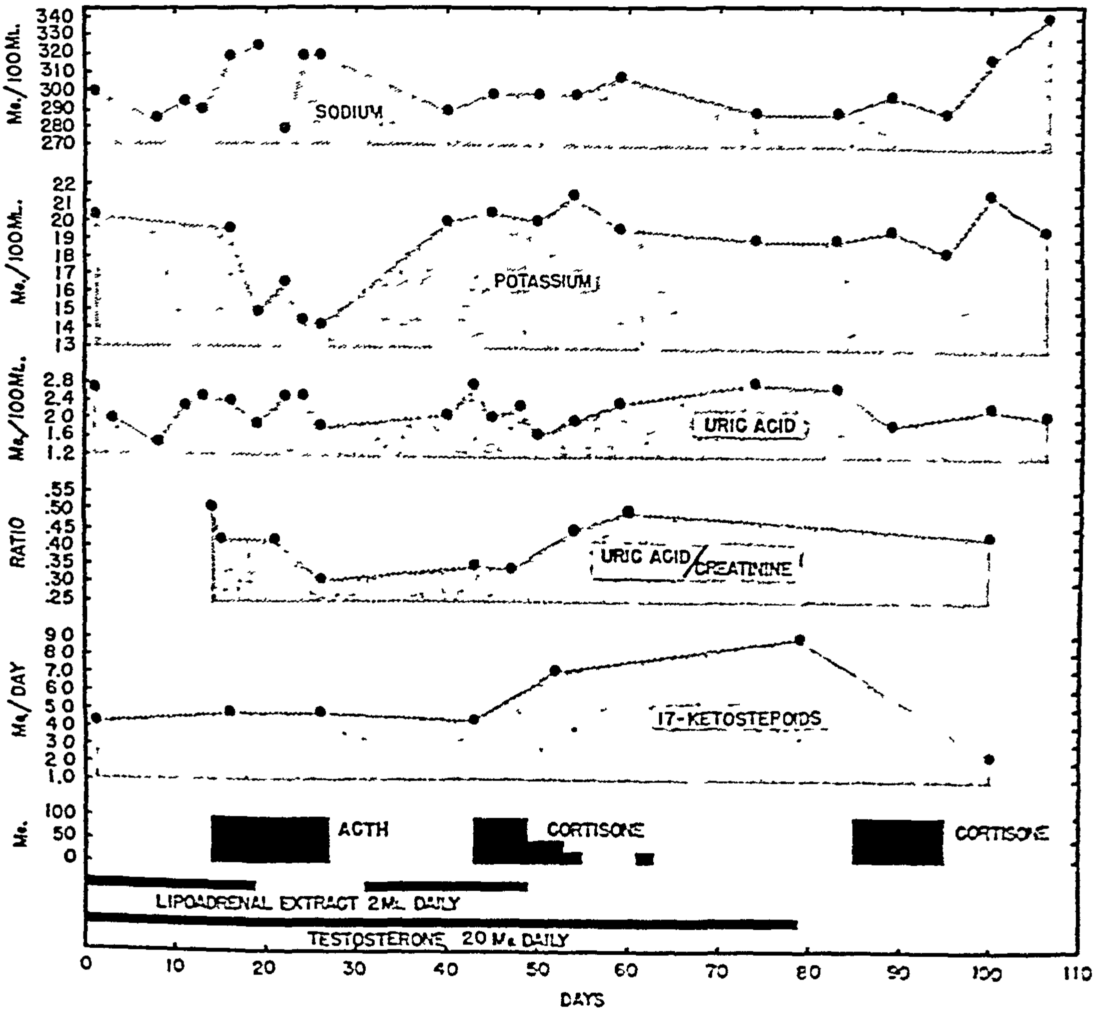

FIG. 3

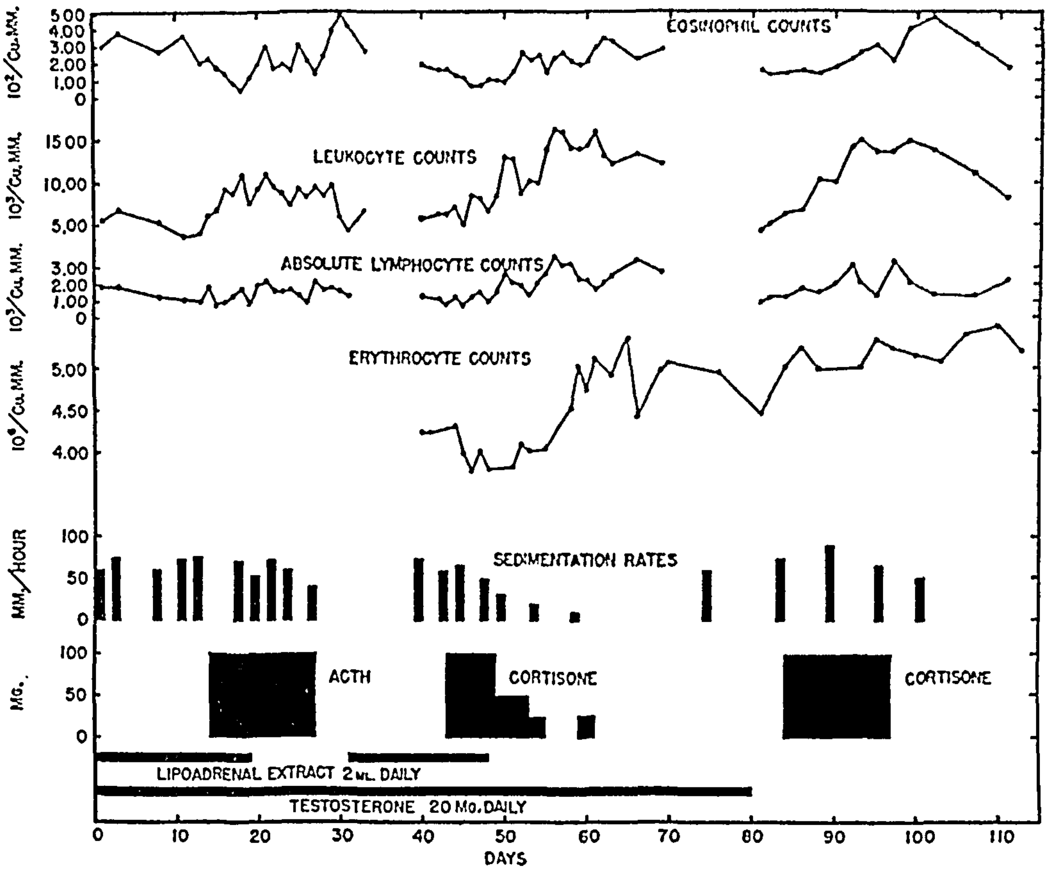

FIG. 4

lipoadrenal cortex (Figure 2). During the first course of cortisone, blood pressure rose from an average control level of 110/80 to 138/98 on the 12th day, concomitantly with the appearance of edema. When cortisone was given without lipoadrenal cortex, testosterone propionate and sodium chloride, only slight elevation of systolic and diastolic arterial tension was noted. Blood pressure did not return to control levels immediately after stopping cortisone, but dropped gradually over a period of 14–21 days.

*Chemical observations:* The data for serum sodium, potassium, uric acid, and 17-ketosteroids are given in Figure 3. Serum sodium varied from 280 to 335 mg./100 ml. with no significant correlation to drug administration. Serum potassium levels dropped to 14.9 mg./100 ml. on the fifth day of ACTH and remained below control levels during the entire course. This was interpreted as suggestive evidence of adrenal cortical activity inasmuch as potassium diuresis had been an observed effect of ACTH administration (3, 7, 8). No alteration of significance occurred during cortisone administration. Neither ACTH nor cortisone significantly altered the blood uric acid levels. The urinary uric acid/creatinine ratio was not significantly altered at any time in the course of this study.

While on testosterone propionate, 20 mg. daily, before and during the control period, 17-ketosteroid excretion ranged from 4.4 to 6.6 mg./24 hours. These figures are somewhat less than the percentage excretion of administered testosterone observed by Fraser, Forbes, Albright, Sulkowitch and Reifenstein (16). While ACTH was administered, no appreciable change in 17-ketosteroid excretion occurred. During the first course of cortisone, while testosterone was continued, an increased excretion of 17-ketosteroids was observed. This effect was noted 20 days after discontinuation of cortisone, and would suggest continued slow absorption from the site of injection, an observation previously made by Sprague, Power, Mason and Cluxton (17). Following the second course of cortisone during which no testosterone was given, the 17-ketosteroid excretion was maintained near the control level by cortisone.

Alteration in tolerance of carbohydrate was not evidenced by fasting and post-prandial blood sugar determinations.

*Hematologic observations:* Values for erythrocyte sedimentation rate, circulating eosinophils, total leukocytes, absolute lymphocytes, and erythrocytes are plotted in Figure 4. The erythrocyte sedimentation rate decreased from an average control value of 68 mm./hour at the termination of ACTH administration to 40 mm./hour. Significant decrease to 10 mm./hour occurred 16 days after initiation of cortisone therapy. During the second course of cortisone a significant decrease in sedimentation rates failed to occur. Total circulating eosinophils dropped from an average control level of 290/cu. mm. to 44/cu. mm. on the fourth day of ACTH therapy. A gradual return to essentially control level by the seventh day of treatment was noted. A similar decrease in circulating eosinophils was observed during the first course on cortisone, while no definite trend occurred during its second exhibition. The total leukocytes rose from an average control value of 5000/cu. mm. to average treatment values of 9000/cu. mm. on ACTH. A pronounced increase in total leukocyte count occurred after six days of cortisone and continued for eight days after its discontinuation. A similar increase was observed during the second course of cortisone. There was very little change in absolute lymphocyte counts during the entire study.

The erythrocyte count rose from control levels of 4,300,000/cu. mm. to 5,980,000/cu. mm. at the completion of the second course of cortisone.

## DISCUSSION

Because this patient had clinical signs of hypothyroidism and a low basal metabolic rate, she was treated with small amounts of thyroid substance on four occasions. The response was adverse and each time the therapy had to be discontinued. The symptoms which caused the latest admission to the hospital suggested adrenal insufficiency, and although she was hypokinetic and her skin was dry the signs of myxedema were not outstanding. ACTH caused no alteration in the relationship of the clinical signs of adrenal insufficiency to those of hypothyroidism, but when the hypoadrenalism was controlled with cortisone, striking signs of myxedema occurred. Clinically it seemed that partial control of either adrenal or thyroid insufficiency enhanced signs of the untreated endocrine deficiency. That there is a relationship between adrenal and thyroid function has been demonstrated by Soffer, Gabrilove, and Jailer (18). Evidence that this relationship is more than relative is not apparent from our study.

The observations set forth in this study of an unusual clinical combination of rheumatoid arthritis and hypofunction of the adrenal cortex secondary to hypopituitarism are in keeping with the rapidly accumulating data in this field (3–6, 8). In our patient there was clinical and laboratory evidence that some bit of adrenal cortex remained and was responsive to stimulation by ACTH. This response mediated through the adrenal glands was shorter and less intense than the general response to substituted cortisone. Generalizations from this series of studies are not warranted.

## SUMMARY

1. ACTH and cortisone were administered to a patient with longstanding panhypopituitarism and rheumatoid arthritis.

2. Partial adrenal cortical activity was demonstrated during ACTH administration.

3. The effect on the arthritis was equivocal after administration of ACTH, but was definite after administration of cortisone.

4. Clinical, chemical and hematologic data are in agreement with present conceptions of the metabolic and therapeutic properties of ACTH and cortisone.

## ACKNOWLEDGMENTS

We wish to thank Mrs. Frances Jolly for the uric acid and creatinine determinations, Doctor R. B. Gibson for the sodium and potassium determinations and Miss Mary R. Bedford, dietician, for estimating the daily food consumption.

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# THE ANEMIA OF THERMAL INJURY. I. STUDIES OF PIGMENT EXCRETION [1, 2]

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(Submitted for publication September 7, 1950; accepted, November 6, 1950)

Our interest in the metabolism of hemoglobin in patients with thermal injury was stimulated by early observations that (*a*) in spite of multiple whole blood transfusions anemia often developed rapidly in the first 96 hours after injury, and (*b*) in the protracted convalescence of a patient with only a small burn, moderate anemia inevitably ensued. The latter was exceptionally refractory to all except replacement therapy. With these two facts in mind we have undertaken our present study of the burn patient in an attempt to answer the following questions: (*1*) to what extent does hemolysis occur, and (*2*) what information about the anemia of the protracted convalescent stage might a continuous investigation of heme metabolism give us?

Previous work on the same general problem has already been presented by Moore, Peacock, Blakeley, and Cope (1), who demonstrated an increased excretion of urobilinogen in the urine of four patients and an increased excretion in the feces in one patient. Similarly, the British workers, Anderson and Semeonoff (2), described increased excretion of urinary urobilinogen. Others have pointed out the potentiality of increased osmotic fragility and plasma hemolysis (3, 4) early in the post-burn period as well as agglutination and "sludging" of the red cells (5) in the peripheral circulation removed from the site of the injury.

Cartwright and his associates have presented several papers discussing some of the metabolic aspects of the anemia of chronic sepsis, and give data showing low serum iron, elevated serum copper and increased urinary coproporphyrin excretion (6). A few studies are available, notably those of Saifi and Vaughan (7, 8), in which estimations of the urobilinogen excretion in the urine and feces in chronic sepsis have been made. In our experience bacterial contamination and growth on the burned area is always present after the first dressing of third degree burns.

In the investigation of an anemia which appears suddenly over a period of a few days, it would seem of some importance to estimate the amount of the end products of hemoglobin catabolism. The correlation of the amount of hemoglobin end products, as urobilinogen, with the total number of grams of circulating hemoglobin has been discussed by Miller, Singer, and Dameshek (9) and others as the hemolytic index, and can serve as a rather reliable method of estimating intravascular hemolysis. Moore and his colleagues (1) in their review mention the initial hemolytic phase of burns but only support this observation with one case with fecal urobilinogen study. We believe the urine urobilinogen may have a different interpretation than that described.

## METHODS AND MATERIAL

Seventeen burn patients admitted to the Burn Service of the Department of Surgery at the Medical College of Virginia were selected for study. The burns were usually of the free fire type caused by kerosene explosions in wood stoves and by blazing clothing. Immediately after admission the patients were started on study with an initial hemogram, a blood volume determination, and a continuous collection of all urine and feces. Subsequent blood studies were performed as indicated. Treatment in all the cases was essentially the same, employing pres-

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[1] Presented in part before the Clinical Congress, American College of Surgeons, October 16–23, 1949, Chicago, Illinois.

[2] Supported by grants to the Surgical Research Laboratory, Department of Surgery, from the Medical Research and Development Board, Department of the Army (Project: S.F. No. W–49–007–MD–411).

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TABLE I

|  | Patient | Age | Sex | Race | Amount of burn in per cent |  | Cause | Result |
|---|---|---|---|---|---|---|---|---|
|  |  |  |  |  | Third degree | Total |  |  |
| Group I<br>0 to 10% | C. W. | 17 | M | W | 0 | 35 | Gasoline explosion | R* |
|  | A. D. | 13 | F | W | 1.5 | 23 | Free fire | R |
|  | R. G. | 28 | M | W | 2.0 | 12 | Electrical | R |
|  | G. C. | 10 | M | W | 4.0 | 70 | Free fire | R |
|  | J. B. | 35 | M | N | 4.0 | 16.5 | Hot water | R |
|  | W. B. | 11 | M | N | 7.0 | 11.0 | Free fire | R |
| Group II<br>11% to 20% | B. C. | 60 | M | W | 11.5 | 22.5 | Free fire | R |
|  | A. C. | 19 | M | N | 12 | 17.5 | Gasoline | R |
|  | B. R. | 15 | M | N | 15 | 20 | Free fire | R |
| Group III<br>21% or greater | C. T. | 31 | M | N | 30 | 47 | Free fire | D* |
|  | F. T. | 33 | M | N | 30 | 95 | Gasoline explosion | D |
|  | J. M. | 84 | M | N | 38 | 48 | Free fire | D |
|  | S. L. | 57 | F | W | 20 | 70 | Free fire | D |
|  | G. L. | 19 | F | N | 51 | 63 | Free fire | D |
|  | F. J. | 20 | F | N | 55 | 65 | Free fire | D |
|  | O. B. | 29 | F | N | 65 | 74.5 | Free fire | D |

* R = Recovered; D = Died.

sure bandage without debridement, parenteral antibiotics, fluids and when indicated whole blood transfusion.

The hematologic methods were those given by Wintrobe (10). Hemoglobin was measured by the alkaline hematin method in sodium carbonate on the Coleman Junior Spectrophotometer, standardized for hemoglobin by the oxygen capacity method. The urine was collected in brown glass bottles, using 5 Gm. of sodium carbonate with petroleum ether as a preservative. Feces were collected in waxed carton containers. Urine urobilinogen determinations were done on one day or two day collections, fecal urobilinogen on four day or longer collections by the quantitative method of Schwartz, Sborov, and Watson (11). We have used 200 mg. per day as the upper limit of normal for fecal urobilinogen studies since this was the highest value obtained on two normal subjects in our laboratory. This is somewhat lower than the upper limits usually described as 250 mg. per day. Total urinary coproporphyrin was determined on the Klett photofluorometer model 1040 by the method of Schwartz, Hawkinson, Cohen, and Watson (12). Standardization of our instrument was performed with coproporphyrin dimethyl ester, prepared, isolated, and purified in our laboratory from porphyria urine and urine from lead poisoned patients. Serum bilirubin (13), T-1824 plasma volume (14), and $P^{32}$ red cell volume (15) were determined at intervals designed to aid us in our studies. The hemolytic index, a ratio of urobilinogen excretion per day to total circulating hemoglobin, has been described by Miller, Singer, and Dameshek (9). It is calculated in the following manner:

$$\text{hemolytic index} = \frac{\text{urobilinogen excretion in mg. per day} \times 100}{\text{total circulating hemoglobin in grams}}.$$

The normal range of this index is given as 11–21.

## RESULTS AND DISCUSSION

Table I presents statistical data on our patients who were arbitrarily separated into three groups based on the percentage of third degree burn as estimated by the Lund and Browder values (16). This division is as follows: Group I, 0 to 10 per cent; Group II, 11 to 20 per cent; Group III, 21 per cent or greater.

The results of the fecal urobilinogen studies should be evaluated in the light of previous experience that whole blood transfusions, particularly if the blood has been stored for more than a week, leads in some instances, not only to a slight increase in the serum bilirubin, but also an increase in fecal urobilinogen (17, 18). Also of considerable importance are the recent clinical studies of London (19) and his associates which indicate that at least 11 per cent of the fecal stercobilin[6] in normal persons is derived from sources other than the pigment fraction of hemoglobin. To what extent increases in fecal urobilinogen (*vide infra*) are due to non-hemoglobin sources in our patients cannot be ascertained, and the possibility exists that tissue destruction is contributing tetrapyrrols, due to intracellular breakdown since all cells contain the ferroprotoporphyrin ring.

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[6] The method used in this work measures both stercobilinogen and mesobilirubinogen and the result of the determination is expressed in terms of urobilinogen.

In Group I there were three instances in which fecal urobilinogen was considered to be increased as evaluated by the hemolytic index. In two of these the increase occurred in the first week and was associated with a marked drop in the hemoglobin and increased hemolytic index. We have interpreted this to mean excessive hemolysis. Neither of these patients received transfusions on admission. J. B. (Figure 1), 4 per cent third degree burns, illustrates an example of this response: Laboratory studies indicated a sharp rise in the fecal urobilinogen and a fall in the total circulating hemoglobin of 122 Gm. The hemolytic index increased from 3.8 to 37.4, later returning to within the accepted normal range of 11 to 21.

In a patient with 35 per cent second degree burn (C. W.) there was evidence of a definite hemolytic phase. It would appear, therefore, that hemolysis is not entirely a question of the depth of burn.

In two instances in Group I there is a different response. A. D. (Figure 2) with 1.5 per cent third degree burn, was given a total of 430 Gm. of hemoglobin shortly after admission raising her total circulating hemoglobin from 523 Gm. to 896 Gm., an increase of approximately 72 per cent. This gave rise to an "artificial" polycythemia. Interestingly enough, she did not show any excessive hemolysis as judged by the fecal urobilinogen studies. The hemolytic index of 10 was normal, but there was a moderate rise in the total serum bilirubin. The interpretation of this discrepancy is somewhat difficult. A possible explanation of this may be due to the difficulty in stool collection in this patient. It is noted that the fecal urobilinogen covers a period of eight days, whereas if the total amount of urobilinogen excreted during this period were computed on a three day basis, which it more nearly represents, the concentration of fecal urobilinogen in mg. per day would be correspondingly higher, and thus the hemolytic index. In addition as it has been pointed out by Ham and his associates (4), thermal

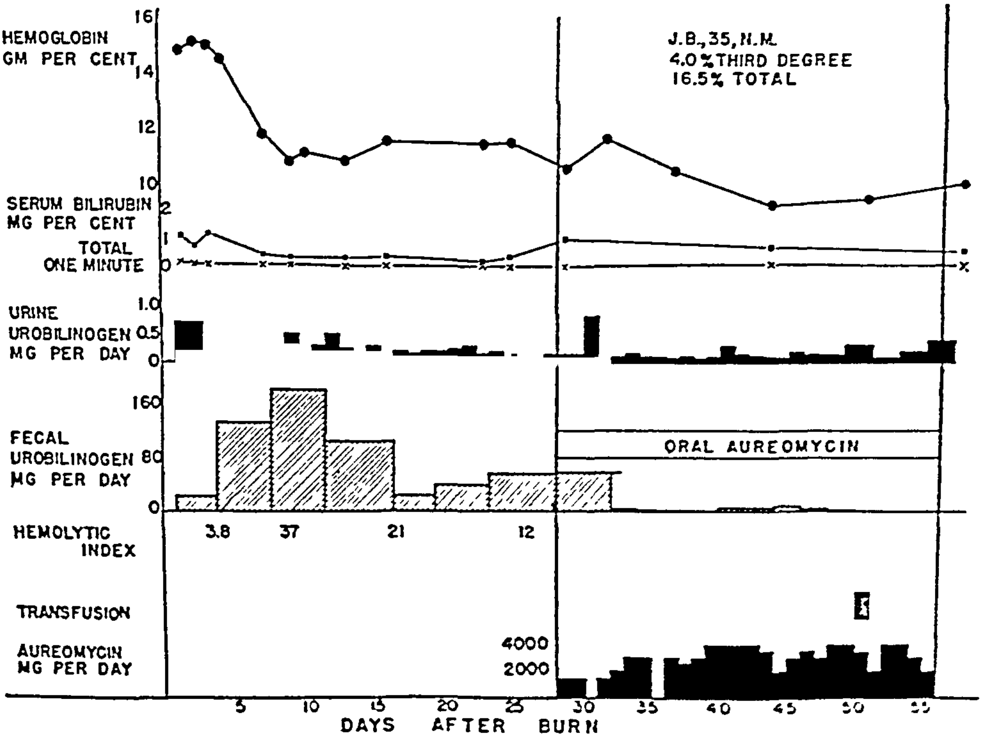

FIG. 1. (J. B.)

The graph demonstrates a drop in hemoglobin with a concomitant rise in the fecal urobilinogen and hemolytic index. Serum bilirubin and urine urobilinogen were unchanged. After the 28th day oral aureomycin was administered. Note the marked diminution in fecal urobilinogen output, without significant change in the urine urobilinogen. The hemolytic index after the 25th day was at the lower limit of normal and suggests decreased breakdown of heme pigments.

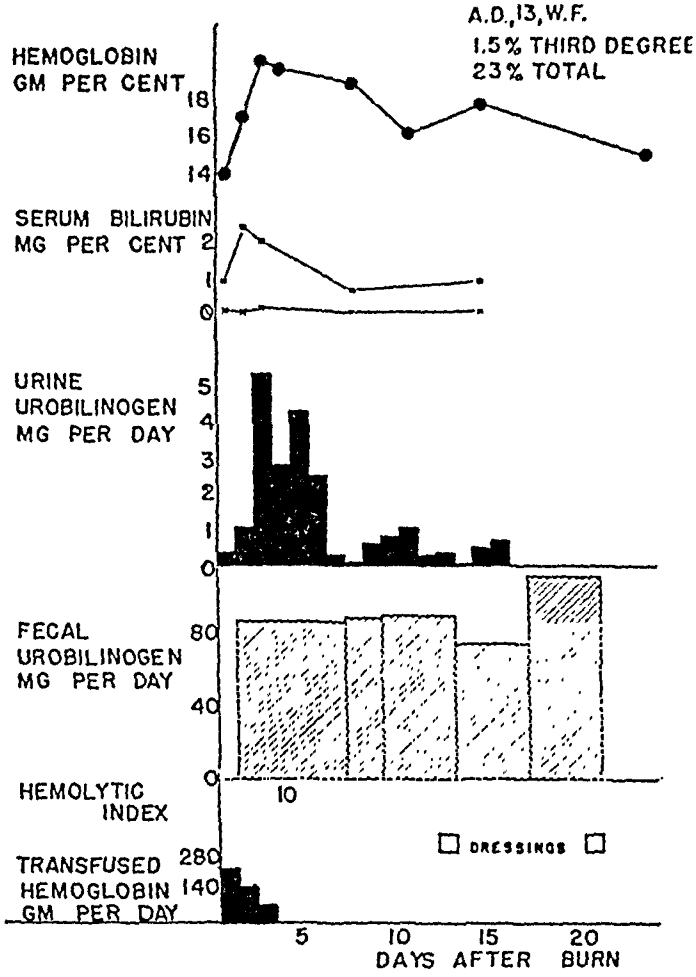

FIG. 2. (A. D.)

This patient was given 430 Gm. of hemoglobin as red cells in the first three days after admission. An artificial polycythemia was produced, but she did not exhibit any increase in the hemolytic index or fecal urobilinogen. On the third post burn day, however, the urine urobilinogen was increased, and there was a rise in the total serum bilirubin on the second day. Increased urine urobilinogen in the presence of a normal fecal urobilinogen suggests impairment of the ability of the liver to properly handle reabsorbed urobilinogen.

trauma leads to red cells potentially more fragile to osmotic and mechanical influences. The transfusion in this patient could give rise to two populations of red cells, the patient's, which are potentially subject to hemolysis, and the transfused cells, not fragile. At the same time the total circulating hemoglobin would have been raised. Hence on the basis of these speculations, if the hemolytic index were recalculated using the initial total circulating hemoglobin (prior to transfusion), the fecal urobilinogen on a three day basis, the index would have been 40. This of course fits well with the other findings of increased indirect serum bilirubin, and the data on the subsequent patients. The other patient who showed a different response was a youth of 10 years with a 4 per cent third degree burn. Although blood volume studies were not done, he did not show a significant rise in the fecal urobilinogen until the 10th day, when the fecal urobilinogen rose to 165 mg. per day.

In Group II are those with third degree burns covering from 11 to 20 per cent of the body surface. The fecal urobilinogen studies are altered by the use of oral or intravenous aureomycin, which will be discussed below.

In the severe group of burns, all of whom expired, there were marked increases in the fecal urobilinogen. Stools could not be collected in all instances but in those patients from whom specimens were obtained increases were all greater than 500 mg. per day, which is in the accepted range of the hemolytic anemias. In one instance, we were able to follow a man, F. T. (Figure 3), with 30 per cent third degree and 95 per cent total burn for a period of 33 days. At no time did his fecal urobilinogen return to normal even during a period when no transfusions were administered, and the hemolytic indices were constantly elevated. This indicates in this particular burn that continuous hemolysis played a significant part in the mechanism of the anemia. From the graph it can be seen that there was a constant and steady decline in the hemoglobin value despite multiple transfusions.

Table II gives a composite picture of the chief results as regards the fecal urobilinogen. In so far as the fecal urobilinogen is concerned, we interpret these data in the following manner. There is usually evidence of increased hemoglobin breakdown in third degree burns of less than 7 per cent of the body surface. In larger burns there is a greatly increased breakdown of hemoglobin as judged by the high excretions of fecal urobilinogen.

The urine urobilinogen appeared to follow a fairly distinct pattern in nearly all patients in the three groups unless oral aureomycin was administered. This consisted of an early rise to abnormal values, usually within the first two or three days. In the group of severely burned patients this substance attained very marked concentrations in the urine as shown in Table II. The urine urobilinogen values are tabulated as the highest one observed during hospitalization, and the value obtained on the third day after the burn. Group III

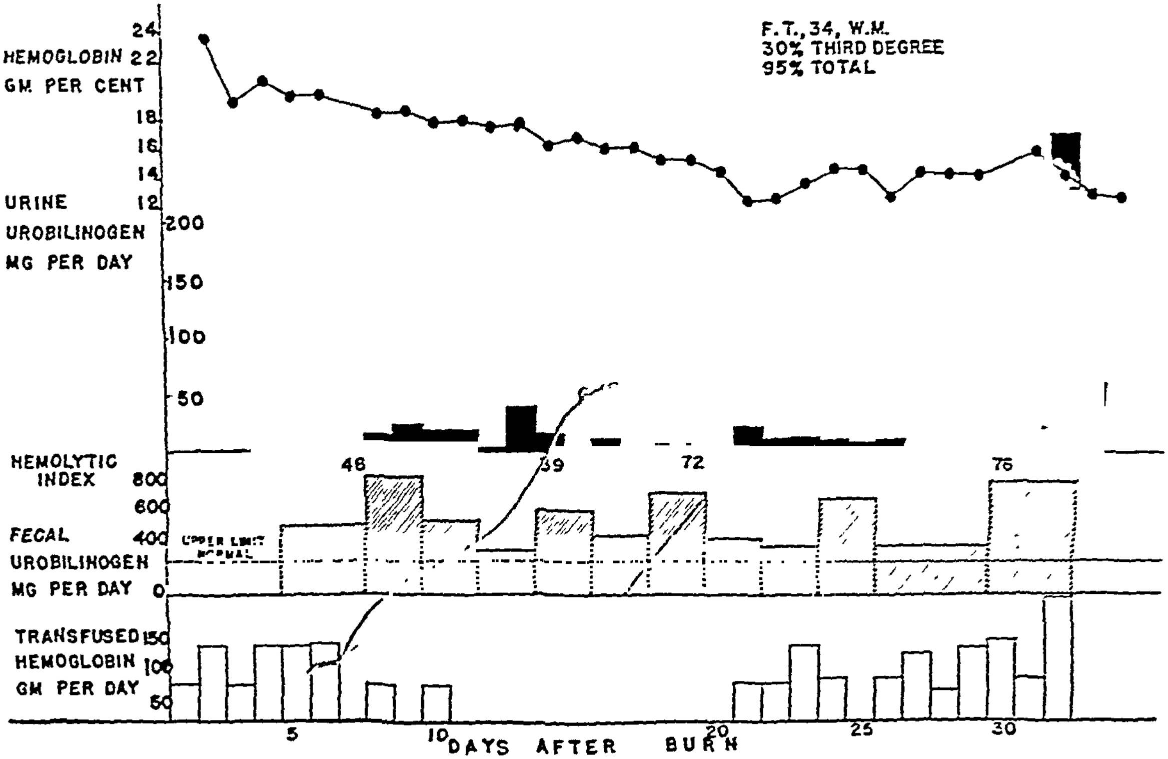

FIG. 3. (F. T.)

This patient with a severe burn demonstrated continuous and marked excretion of fecal urobilinogen and enormous increase in the urine urobilinogen immediately after injury and just before death. Despite daily transfusions except between the 11th and 20th days his hemoglobin dropped steadily, likewise during this same period the fecal urobilinogen remained elevated, and the hemolytic index was continuously increased.

TABLE II

*Urine and fecal urobilinogen, hemolytic index, and serum bilirubin*

| | Patient | Amount of burn | | Urine urobilinogen (*mg. per day*) | | Fecal urobilinogen | | Serum bilirubin | | Hemolytic index | Aureomycin |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Third degree | Total | Highest | Third day post burn | (*mg. per day*) | Post burn day | 1' | Total | | |
| Group I | C. W. | 0 | 35 | 18 | 18 | 300 | 6 | 0.12 | 2.4 | 34.2 | No |
| | A. D. | 1.5 | 33 | 5.4 | 5.4 | 90 | 6 | 0.2 | 2.4 | 10.1 | No |
| | R. G. | 3.0 | 12 | 32.0 | 11.0 | 215 | 6 | — | — | — | No |
| | G. C. | 4.0 | 70 | 8.2 | 1.5 | 90 | 4 | 0.2 | 0.8 | — | No |
| | J. B. | 4.0 | 16.5 | 1.2 | 0.3 | 180 | 8 | 0.1 | 1.1 | 37.4 | No |
| | W. B. | 7.0 | 7.0 | 20 | 3.4 | 150 | 4 | 0.4 | 0.85 | 49.7 | No |
| Group II | A. C. | 11.5 | 22.5 | 42 | 37.0 | —* | — | — | — | — | No |
| | B. C. | 12.0 | 17.5 | 0.2 | 0.1 | 0.6 | 2 | 0.32 | 4.05 | — | Yes |
| | B. R. | 15.0 | 20 | 6.0 | 6.0 | 10.6 | 4 | 0.16 | 0.23 | — | Yes (I.V.) |
| Group III | C. T. | 30 | 42 | 250 | 250 | 530 | 5 | 0.2 | 2.3 | — | No |
| | F. T. | 30 | 95 | 275 | 45 | 800 | 5 | 0.6 | 1.2 | 37 | No |
| | J. M. | 38 | 48 | 157 | 6.0 | 582 | 11 | 0.4 | 1.9 | 54 | No |
| | S. L. | ? | 70 | 67 | 11 | —§ | — | 1.34 | 2.17 | — | No |
| | G. L. | 51 | 63 | 32 | 14 | 23† | 4 | 4.6 | 7.55 | — | No |
| | F. J. | 55 | 65 | 0.15 | 0.1 | 6.3‡ | 12 | — | — | — | Yes |
| | O. B. | 65 | 75 | 0.8 | 0.7‡ | — | 3 | 1.7 | 5.39 | — | Yes |

* Diarrhea incomplete collection.
† Only 40 gm. of feces obtained.
‡ No feces first nine days.
§ Feces lost.

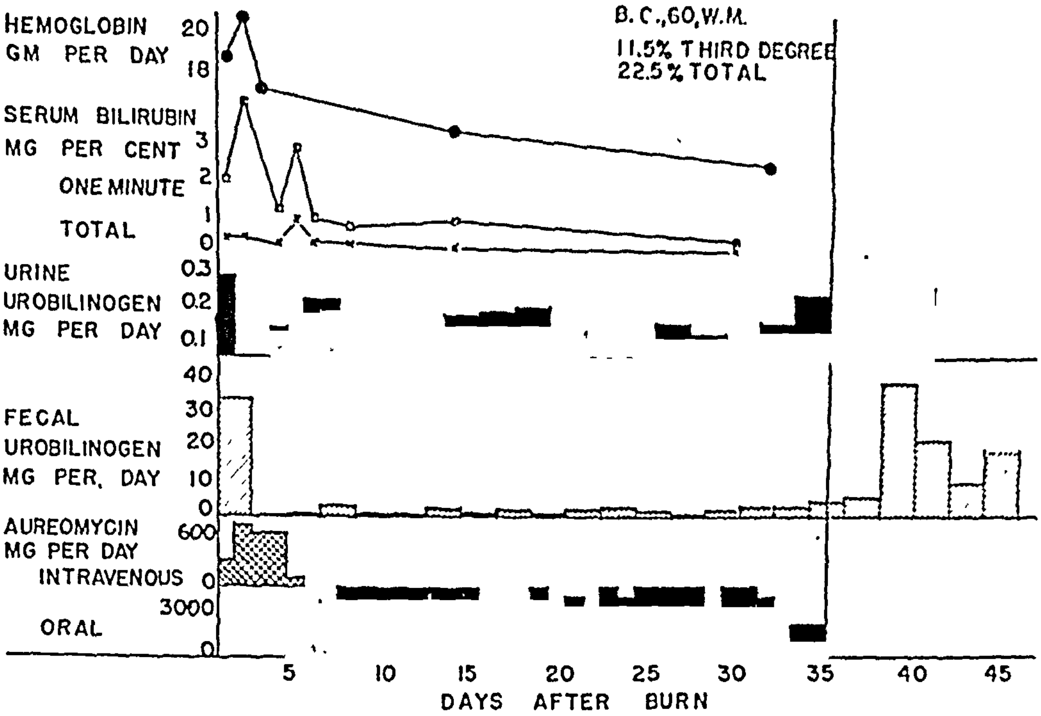

FIG. 4. (B. C.)

This demonstrates the effect of aureomycin on the fecal urobilinogen from the time of admission to the 35th hospital day. Oral aureomycin was not begun until the fifth post burn day, and the effect of intravenous aureomycin during the early period is equivocal. Note the drop in the hemoglobin and the rise in the serum bilirubin. Hemolytic indices could not be run because of the aureomycin effect.

patients show the most marked concentrations, but elevations are present in all except those receiving aureomycin and in one patient with uremia and almost complete anuria. Both Figures 2 and 3, are representative examples of the changes seen in our patients. This increased excretion of urobilinogen in the urine we interpret as evidence of early and rather marked hepatic failure, failure to properly catabolize the portal blood urobilinogen. Hence it appears in the peripheral blood and is excreted in the urine. This subject is to be dealt with more fully in a later paper.

One patient in Group I, J. B. (Figure 1); two patients in Group II, B. R. and B. C. (Figure 4); and two patients in the severely burned group, F. J. and O. B., were given either oral or parenteral aureomycin, or both, to observe the effect of this antibiotic on the excretion of urobilinogen in the urine and stool.

Briefly, bilirubin derived from hemoglobin is reduced by bacteria in the gastro-intestinal tract to mesobilirubinogen and stercobilinogen, both colorless compounds and conveniently grouped together with the name of urobilinogen because of an identical chromogenic reaction with Ehrlich's reagent. These are apparently entirely of enterogenous origin. Reabsorption of the urobilinogen takes place in the gut, and the liver effectively removes or catabolizes these substances in such a manner that only a small amount, up to 3 mg. per day (actually in our experience only 1.5 mg. per day), is excreted in the urine.

Sborov and his associates have already discovered the effects of aureomycin on fecal urobilinogen due to its sterilizing effect on the fecal flora (20). Our experience in finding a marked reduction is nearly the same as theirs. It is interesting to record, however, that petroleum ether-soluble Ehrlich-reacting substances, presumably tetrapyrroles, were still present. These effects with aureomycin [7] are shown graphically in Figures 1 and 4.

B. R. (Table II) who suffered about 15 per cent third degree burn received both intravenous and oral aureomycin from the onset but severe nausea and vomiting make it uncertain as to how much oral aureomycin was retained. We observed that no increase of the fecal urobilinogen occurred, but a significant rise in urine urobilinogen was evident. After aureomycin was stopped, and following a transfusion there was a sharp rise in fecal urobilinogen. The patient B. C. (Table II) with 12 per cent third degree burn, received intravenous aureomycin initially with equivocal effect on the fecal urobilinogen. After the administration of oral aureomycin the amount of Ehrlich-reacting substances was less than 5 mg. per day. Likewise the urine urobilinogen was exceedingly low giving values of 0.1 to 0.2 mg. per day. Four days after discontinuing aureomycin the fecal urobilinogen rose to values which we have observed commonly in the convalescent phase of the moderately burned patient. The fecal urobilinogen values were extremely low in a previous patient discussed (J. B., Figure 1), ranging between 0.6 and 6 mg. per day during the time when he was receiving oral aureomycin.

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[7] The aureomycin for these studies was generously supplied by the Lederle Laboratories Division, American Cyanamid Company.

As shown previously there is, in the severe group, a marked increase in the fecal urobilinogen, but in one patient, F. J., with 55 per cent third degree burn who received aureomycin, and in whom other evidence of hemolysis was present, we observed the fecal urobilinogen did not increase, and the amount of Ehrlich-reacting compounds remained essentially the same, *i.e.*, less than 5 mg. per day and usually about 1.2 mg. per day. Likewise, the urine urobilinogen in this patient was extremely low ranging from 0.1 to 0.2 mg. per day. These observations naturally raise the question of whether the Ehrlich-reacting substance present after aureomycin is a urobilinogen, since it is rather constant even when the total amount of petroleum ether-soluble Ehrlich-reacting compounds varies over a wide range.

The total urinary coproporphyrin excretion was studied in six patients who are in the following categories. Two in Group I, three in Group II, and one in Group III. Since this determination was not begun until late in the study, it does not include all patients. There was an increased excretion of total urinary coproporphyrin in all patients studied, and it did not appear to vary with severity of the burn. In four of these, normal

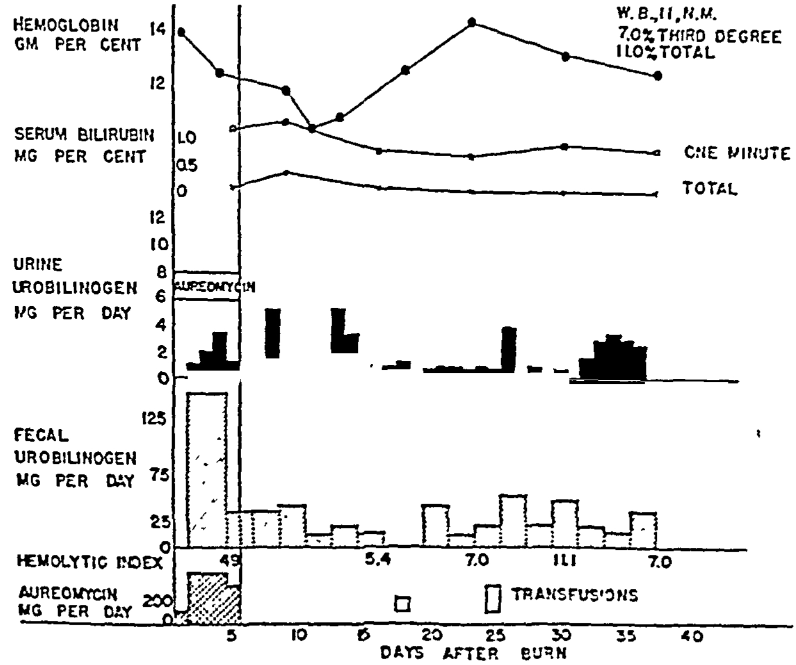

FIG. 5. (W. B.)

In this patient fecal urobilinogen was initially increased and intravenous aureomycin had no effect on either the fecal or urine urobilinogen excretion. Note the increased hemolytic index with a concomitant drop in hemoglobin. After two weeks and during convalescence the hemolytic index was low suggesting decreased heme breakdown.

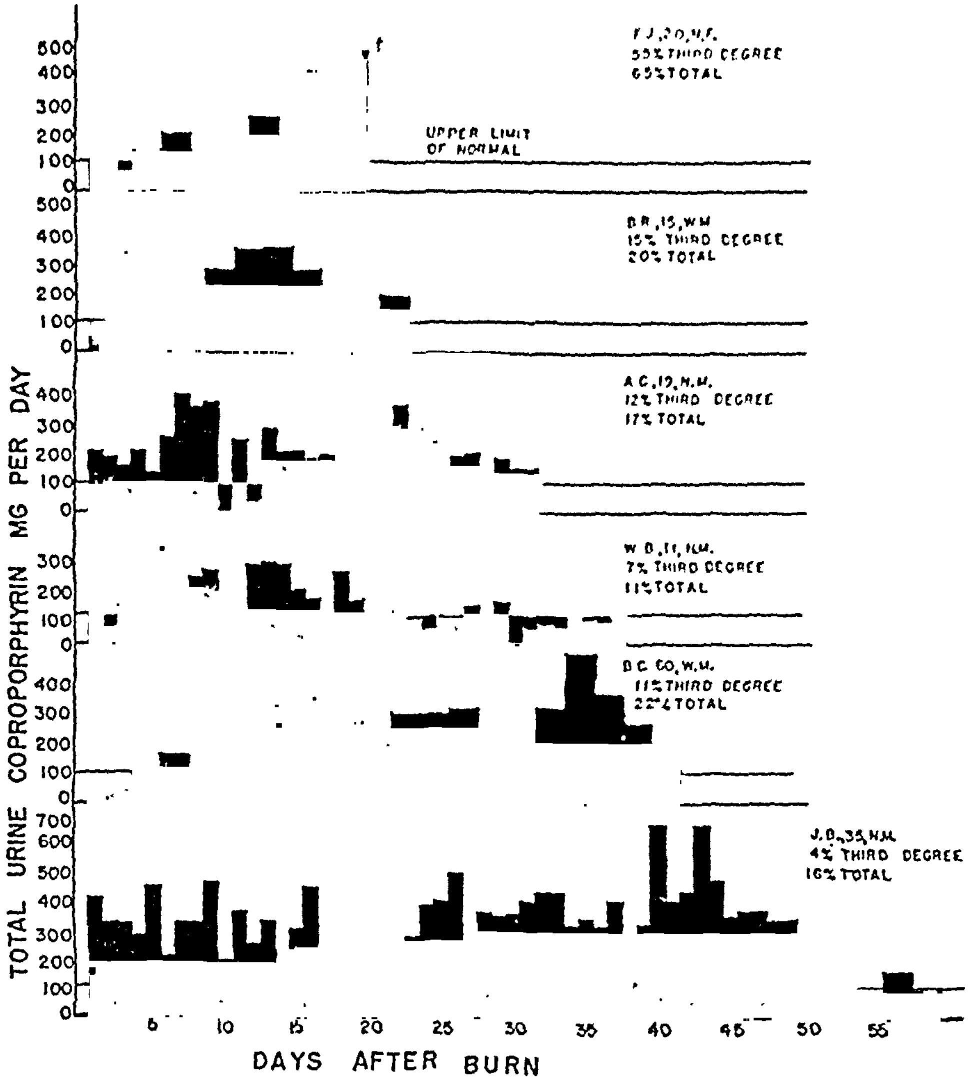

FIG. 6.

Urine coproporphyrin excretion was increased in six patients who showed varying amounts of third degree burns. High initial values were observed in two patients, A. C. and J. B. Aureomycin was administered to five of these patients with no observable effect on the coproporphyrin excretion, which returned to normal or near normal in five patients as healing was complete. F. J. expired on the 19th day.

values obtained upon admission became greatly elevated. In two others the values were greater than normal on admission and, therefore, may have resulted from some cause other than the burn. But in these patients the concentration increases over the admission value. Figure 6 shows the composite graph of the urine coproporphyrin in all patients. It was noted that in five of these patients (J. B., B. C., W. B., F. J., B. R.), oral aureomycin had no effect on the coproporphyrin excretion. Isomer analysis was not performed on any of these urines so that distribution of types cannot be determined. It would appear that the excretion of urinary coproporphyrin is the greatest during the convalescent phase and remains elevated until healing is almost complete.

Our study would make it appear that in the severely burned patient hemolysis must always be considered in the evaluation of the anemia and in the treatment of the early postburn phase. Even in the less severely burned patient hemolysis takes place. Brooks and his colleagues have demonstrated sludging of the red cells with subsequent agglutination and phagocytosis (*21*). Red cell destruction is associated with the opening of the porphyrin ring and subsequent degradation of hemoglobin to open chain tetrapyrroles. In our patients the drop in hemoglobin, the rise in hemolytic index, and often a rise in the indirect type of serum bilirubin are other experimental data to support this concept. In one severely burned patient there was evidence of hemolysis from admission to demise 32 days later.

In the study of the burn patient during convalescence and grafting, the ratio of the fecal urobilinogen to the total circulating hemoglobin would seem to point to decreased hemolytic activity, which may result from decreased hemoglobin synthesis. Actually the hemolytic index is lowered (W. B., Figure 5). Increased coproporphyrin excretion may also be a reflection of a disturbance in hemoglobin synthesis.

The synthesis component of the anemia of burns has been very thoroughly discussed by Moore and his associates (1) and they have used the method of estimating the uptake of radioactive iron as a guide in evaluating the marrow depression. One study showing a low fecal urobilinogen excretion, and hence hemoglobin catabolism, with low hemolytic indices in the convalescent phase, suggests a decreased breakdown (Figure 5). We believe that one of the most important factors in the apparent marrow depression is the presence of bacterial growth on the burned area. The urinary coproporphyrin studies during the convalescent phase may favor this idea. However, the urinary coproporphyrin findings may be evidence of liver malfunction.

Moore and his colleagues (1) described increases in urine urobilinogen in their patients, and our data is in agreement with their work. We interpret differently the increased quantity of urine urobilinogen even though there is an increased amount of fecal urobilinogen in the stool. The level of the urinary urobilinogen is probably a very sensitive index of liver cell function. In burns, what we are measuring by the urobilinogen excretion is not only evidence of hemolysis but also impairment of the ability of the liver to properly catabolize the increased amounts of this substance.

The present studies would seem to answer the question concerning the occurrence of hemolysis. We believe that hemolysis occurs extensively, particularly in the large burn, and must always be reckoned with in evaluating these patients. This study has not answered the second question entirely to our satisfaction. A continuous investigation of heme catabolism shows us there is a decreased breakdown of heme products during the convalescent phase of burns. It gives us very little insight into the mechanisms involved in this process. In severely burned patients, during the long convalescent phase there may be considerable hemolysis taking place as evidenced by an increased hemolytic index and an increased fecal urobilinogen. Increased breakdown coupled with a depressed bone marrow would make the anemia more severe. As our work has indicated in one patient, in spite of a repeated transfusion, anemia progressed rapidly.

No conclusion can be drawn about the effect of aureomycin as far as anemia of thermal burns is concerned, but we might draw some interesting speculation about urobilinogen metabolism and Ehrlich-reacting substances in the feces in patients who are receiving oral aureomycin. It would appear that aureomycin can effectively sterilize the gastro-intestinal tract, so that at least the main reduction agents of bilirubin are inhibited to such an extent that little or no reduction of bilirubin occurs. We, nevertheless, consistently found in the feces a small amount of Ehrlich-reacting substances. Whether these small quantities are separate and entirely different compounds from urobilinogen we are unable to say. We are tempted to think they may be other substances since, in patients in whom we expect large increases of urobilinogen, the detectible quantity of these compounds remains almost the same as that in patients who are excreting only an expected small amount of urobilinogen.

## CONCLUSIONS

1. Hemolysis, as judged by fecal urobilinogen excretion compared to total circulating hemoglobin, occurs in burns of all degrees. It is very great in third degree burns of more than 20 per cent.

2. There is usually a constant increase in the urinary urobilinogen noted on the third day after injury and this probably denotes early hepatic dysfunction and inability to properly catabolize an increased amount of urobilinogen.

3. Oral aureomycin effectively reduces the fecal and urinary urobilinogen to very small amounts. It is questioned whether the petroleum ether-soluble Ehrlich-reacting substances found after oral aureomycin administration are urobilinogens or whether they are entirely different compounds.

4. Additional evidence is presented to show that the anemia of thermal burns is at first hemolytic, then dyshemopoietic.

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# THE ANEMIA OF THERMAL INJURY. II. STUDIES OF LIVER FUNCTION [1,2]

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(Submitted for publication September 7, 1950; accepted, November 6, 1950)

In a previous communication it was demonstrated that an increased excretion of urine urobilinogen was an early and constant finding in thermal injury even in burns of a relatively small amount of third degree (1). Since this particular finding was interpreted by us as a manifestation of hepatic injury, we undertook the further study of certain liver function tests in burned patients, none of whom received sulfonamides, tannic acid or other escharing therapy.

Bardeen published a review of the pathology of severe burns in 1899 and noted at that time changes in the liver and the kidney (2). Wolff, Elkinton and Rhoads demonstrated in three burn patients B.S.P. retention, slight icterus, and a decreased glucose tolerance as well as a decreased hippuric acid excretion test for liver function (3), and Boyce records similar observations and remarks upon the manifestation of jaundice in 65 per cent of the burn patients reported by him (4).

The histopathology of liver changes from 21 to 57 hours after injury has been described as fatty metamorphosis of the epithelial cells in the central zone of the hepatic lobules, and nuclear damage, especially karyolysis. After 57 hours there is slight enlargement, the liver is lighter yellow, greasy, friable, and has venous congestion of the "nutmeg" type. In addition there is evidence of degeneration and necrosis (5). It was believed that the central rather than the peripheral zone was first affected. Gillman and Gillman examined the livers of 38 patients dying within one hour and up to 36 days after burns and showed fatty changes and atrophy of the cells in the central zone. Eight days seemed to be necessary for the development of a very fatty liver, and it would appear that tannic acid intensified the tendency toward necrosis, which they believe present in some patients with burns. However, the majority of the microscopic liver changes were insufficient to account for a fatal termination. They concluded that if the liver contributed to death it did so through altered biochemical effects. It was the opinion of some that tannic acid therapy was responsible for precipitating liver damage (6) and the liver damage from tannic acid was central in character (7). Duffin reports an autopsied case with severe liver failure (8). Hepatic necrosis by his standard occurred in only six patients. Baker made an analysis of 87 autopsied burns on whom liver sections were available. In 56 death occurred before the sixth day. In only three of these was necrosis sufficient to involve nearly one-half the liver lobule, and all of these succumbed on the eighth day after burning. None received tannic acid. He concludes that minimal hepatic necrosis may apparently be caused by burns. Histologically demonstrable fat was frequently seen in those patients dying in the first 24 hours, and in those dying after four weeks. He concludes that necrosis of the liver is not a lesion characteristic of burns (9).

Hartmann and Romence studied liver damage in dogs and found gross enlargement of both the liver and spleen (10). In acute experiments of four to ten days, histopathology demonstrated hepatic lesions ranging from marked congestion of the sinusoids and compression of the liver cords

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[1] Presented in part before the Clinical Congress, American College of Surgeons, October 16–23, 1949, Chicago, Illinois.

[2] Supported by grants to the Surgical Research Laboratory, Department of Surgery, from the Medical Research and Development Board, Department of the Army (Project: S.F. No. W-49-005-MD-411).

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to varying amounts of granular, vascular, and fatty degeneration. Central necrosis occurred in eight to ten days, and liver function was depressed. They did not find the picture of acute liver insufficiency. Belt (11) believes the liver necrosis following burns simulates the lesions of yellow fever and described Councilman bodies and nuclear inclusion bodies. His patients had tannic acid treatment.

### METHODS AND MATERIALS

Fifteen burn patients admitted to the Burn Service of the Department of Surgery of the Medical College of Virginia were selected for study. The majority of the burns were usually of the free fire type from kerosene explosions on wood stoves or blazing clothing. Blood for liver function tests was taken on admission and repeated at intervals during the course of the treatment. In some instances only one set of determinations could be obtained because of early demise or difficulty in obtaining blood. Treatment in all cases was essentially the same and employing pressure bandages without debridement. Parenteral antibiotics, whole blood and replacement fluid therapy were used as needed clinically. No tannic acid, escharing agent, or sulfonamides were employed. In some instances the patients during the course of study were on a special liquid diet providing 100 Gm. of protein, with sufficient fat and carbohydrate to give an average of 45 Calories per kilogram, and vitamin supplements as recommended by the National Research Council.

The following liver function tests were performed: Bromsulfalein retention using 5 mg. per kilogram and taking a 45 minute sample (12); cephalin-cholesterol flocculation at the modified pH of 7.4 (13); thymol turbidity (14); prothrombin time employing the one stage method of Quick (15); total protein and albumin-globulin ratio (16); the serum bilirubin employing the Ducci-Watson modification of the Malloy and Evelyn technique (17); and the quantitative urine urobilinogen (18).

As in a previous study the patients were separated into three clinical groups on the basis of the amount of surface area involved by third degree burn. There were five patients in Group I who had burns of from 0 to 11 per cent third degree; three in Group II, 12–20 per cent; and seven whose burns were greater than 21 per cent in Group III.

Autopsy material was obtained on five of the six patients in Group III, all of whom expired.

### RESULTS AND DISCUSSION

Table I gives the composite results of the liver function tests on all 15 patients as performed within the first ten days (usually within the first five days) after the burn. The most constant finding is an increased amount of urobilinogen in the urine, which in one instance reached as high as 75 mg. per day.

In the five patients who had less than 10 per cent third degree burns we see there was bromsulfalein retention of greater than 20 per cent in two of the three patients in whom this test was performed. In four of the five patients there was an elevation of the thymol turbidity test to greater than four units which is considered the upper limit of normal in our laboratory. Two of the four patients in whom the cephalin-cholesterol floccula-

TABLE I

*Composite table of liver function studies*

| | Patient | Age | Sex | B.S.P. retention | Cephalin flocculation | Thymol turbidity | Prothrombin concentration | Urine urobilinogen | Total protein | Albumin | Globulin | Albumin-globulin ratio | Serum bilirubin | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | | 1 min. | total |
| | | | | *per cent* | | *units* | *per cent* | *mg. per day* | *Gm. per cent* | *Gm. per cent* | *Gm. per cent* | | *mg. per cent* | *mg. per cent* |
| Group I | J. B. | 35 | M | 21 | 3 | 7 | 75 | 1.2 | 5.8 | 2.75 | 3.09 | 0.87 | 0.02 | 0.40 |
| | C. W. | 17 | M | 2.7 | 1 | 6 | 43 | 17.0 | 5.9 | 3.7 | 2.2 | 1.68 | 0.24 | 2.52 |
| | W. B. | 11 | M | 32 | 1 | 7 | 60 | 5.2 | 6.95 | 3.1 | 3.85 | 0.80 | 0.4 | 0.85 |
| | G. C. | 10 | M | — | — | 4 | — | 3.8 | 4.8 | 2.6 | 2.2 | 1.18 | 0.25 | 0.45 |
| | A. D. | 13 | F | — | 4 | 2 | — | 6.1 | 6.4 | 3.1 | 3.3 | 0.94 | 0.07 | 2.45 |
| Group II | B. C. | 60 | M | 23 | 3 | 5 | 19 | 0.1 | 6.0 | 3.4 | 3.6 | 0.90 | 0.32 | 4.06 |
| | B. R. | 15 | M | 7.9 | 3 | 3 | 60 | 6.0 | 6.15 | 2.84 | 3.21 | 0.88 | 0.16 | 0.23 |
| | A. C. | 19 | M | 19 | 0 | 6 | 43 | 40.0 | 7.25 | 4.0 | 3.25 | 1.23 | — | — |
| Group III | O. B. | 29 | F | 48 | 0 | 16 | 43 | 0.18 | 5.8 | 2.3 | 3.5 | 0.66 | 1.7 | 5.39 |
| | G. L. | 19 | F | 18 | 1 | 2 | — | 32 | 5.9 | 3.3 | 2.6 | 1.27 | 4.6 | 7.55 |
| | S. L. | 57 | F | 28 | 3 | 4 | 75 | 65 | 6.7 | 2.8 | 3.9 | 0.71 | 1.34 | 2.17 |
| | F. J. | 20 | F | 31 | 3 | 2 | 33 | — | 4.8 | 2.05 | 2.75 | 0.74 | — | — |
| | C. T. | 31 | M | 52.8 | 4 | 1 | — | 250 | 5.5 | 3.1 | 2.4 | 1.29 | 9.02 | 13.85 |
| | F. T. | 31 | M | 19.0 | 4 | — | — | 274 | 4.3 | 2.4 | 1.9 | 1.26 | 0.7 | 1.2 |

TABLE II

*J. B., N. M., 35, 4 per cent third degree burn*

| Date | Days post-burn | B.S.P. retention | Cephalin flocculation | Thymol turbidity | Prothrombin concentration | Total protein | Albumin | Globulin | Serum bilirubin |  | A/G ratio |
|---|---|---|---|---|---|---|---|---|---|---|---|
|  |  |  |  |  |  |  |  |  | 1 min. | total |  |
|  |  | *per cent* |  | *units* | *per cent* | *Gm. per cent* | *Gm. per cent* | *Gm. per cent* | *mg. per cent* | *mg. per cent* |  |
| 10–26 | 1 | 12 |  | 1 | 100 | 6.58 | 3.87 | 2.71 | 0.12 | 1.09 | 1.43 |
| 10–27 | 2 |  |  |  |  | 5.37 | 3.10 | 2.17 | 0.07 | 0.71 | 1.43 |
| 10–28 | 3 |  |  |  | 75 | 5.58 | 2.75 | 2.83 |  |  | 0.97 |
| 11–1 | 7 | 14.4 |  | 5 | 100 | 5.8 | 2.71 | 3.09 | 0.11 | 0.40 | 0.87 |
| 11–3 | 9 |  |  |  |  |  |  |  | 0.11 | 0.18 |  |
| 11–4 | 10 | 21.3 | 3⁻ | 7 | 75 | 6.14 | 3.35 | 2.79 |  |  | 1.20 |
| 11–7 | 13 |  |  |  |  |  |  |  | 0.02 | 0.30 |  |
| 11–10 | 16 |  |  |  |  |  |  |  | 0.01 | 0.46 |  |
| 11–12 | 18 | 19 |  | 1 | 75 | 6.10 | 2.58 | 3.52 |  |  | 0.73 |
| 11–17 | 23 |  |  |  |  |  |  |  | 0.02 | 0.12 |  |
| 11–19 | 25 |  |  | 2 | 50 | 6.50 | 3.6 | 2.1 | 0.07 | 0.46 | 1.16 |
| 11–23 | 29 | 5.4 |  |  |  |  |  |  | 0.05 | 1.03 |  |
| 11–28 | 35 |  |  | 2.5 | 60 | 6.24 | 3.1 | 3.14 |  |  | 0.98 |
| 11–30 | 37 |  |  |  |  |  |  |  | 0.18 | 0.55 |  |
| 12–1 | 38 | 22.8 |  |  |  |  |  |  | 0.14 | 0.56 |  |
| 12–3 | 40 |  |  | 2 | 75 | 5.6 | 2.84 | 2.76 |  |  | 1.03 |
| 12–8 | 45 | 25.0 |  |  |  |  |  |  | 0.15 | 0.71 |  |
| 12–10 | 47 |  |  | 2 | 60 | 5.6 | 2.6 | 3.0 |  |  | 0.86 |
| 12–15 | 52 |  |  |  |  |  |  |  | 0.10 | 0.28 |  |
| 12–16 | 53 | 10.9 |  |  |  |  |  |  |  |  |  |
| 12–17 | 54 |  |  | 2 | 38 | 6.20 | 3.35 | 2.85 |  |  | 1.17 |
| 12–22 | 59 | 4.6 |  | 3 | 60 | 5.8 | 2.84 | 2.96 | 0.11 | 0.56 | 1.15 |

tion tests were done showed elevated values. Total serum proteins were slightly lowered in all except one of these patients, and the albumin-globulin ratios were decreased.

In one patient who was followed for a period of about 60 days, and whose protocol is shown in Table II we see a constant bromsulfalein retention until the time of complete healing. Cephalin flocculation, and thymol turbidity were increased at first, but the thymol turbidity was not abnormal after the tenth post-burn day. In this instance repeated determination of the serum bilirubin was normal, and the urine urobilinogen excretion did not become abnormal at any time, but the urinary coproporphyrin [6] was definitely elevated until just before complete healing was accomplished and the patient discharged. The total protein data showed an initial low normal total protein with a normal ratio of albumin and globulin, but by the seventh day the albumin fraction had decreased so that the globulin was now greater, and there remained a disturbance of this ratio for the next 40 days, despite an adequate protein intake of over 100 Gm. per day with a caloric intake of 45 Calories per kilogram. Identical changes in the total protein and albumin-globulin ratios were observed in the three other patients in this group. The only patient who did not show the reversal of the A/G ratio and no decrease in the total protein was a boy with a 35 per cent first and second degree burn. He also did not show any change in the B.S.P. retention.

In three patients (Table I) who had greater than 10 per cent third degree burn, but less than 20 per cent burn, all showed B.S.P. retention, but in one of them it was only 7.9 per cent. The cephalin flocculation was abnormal in two patients but the changes in the thymol turbidity were not marked. Two patients received aureomycin and in one the urine urobilinogen excretion was modified by this drug. The other, B. R., had an increased excretion of urinary urobilinogen probably because severe nausea and vomiting prevented retention of the oral aureomycin. Table I gives the other data collected for these three patients.

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[6] These data have been presented elsewhere (1)

TABLE III

*B. C., W. M., 60, 11.5 per cent third degree burn*

| Date | Days post-burn | B.S.P. retention | Cephalin flocculation | Thymol turbidity | Prothrombin concentration | Total protein | Albumin | Globulin | A/G ratio | Serum bilirubin | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | 1 min. | total |
| | | *per cent* | | *units* | *per cent* | *Gm. per cent* | *Gm. per cent* | *Gm. per cent* | | *mg. per cent* | *mg. per cent* |
| 12-9-49 | 0 | | | 5 | 75 | 6.1 | 4.4 | 1.7 | 2.58 | 0.3 | 1.95 |
| 12-10 | 1 | 22.3 | 0 | | 60 | 6.0 | 3.4 | 3.6 | 0.9 | 0.32 | 4.06 |
| 12-12 | 3 | | 3+ | 2 | 50 | | | | | 0.91 | 2.89 |
| 12-15 | 6 | 23.1 | | | | 4.8 | 3.1 | 1.7 | 1.82 | | |
| 12-16 | 7 | | | | | | | | | 0.12 | 0.65 |
| 12-17 | 8 | | 0 | 3 | 27 | 5.2 | 2.81 | 2.36 | 1.2 | | |
| 12-20 | 11 | | | | 100 | | | | | | |
| 12-22 | 13 | 13.2 | 0 | 3 | 60 | 4.6 | 2.3 | 2.3 | 1.0 | 0.08 | 0.80 |
| 12-27 | 18 | | 0 | 2 | 50 | 5.0 | 1.6 | 3.4 | 0.47 | | |
| 12-30 | 21 | 12.2 | | | | | | | | | |
| 1-4-50 | 26 | | | | 100 | 5.6 | 1.7 | 3.95 | 0.43 | | |
| 1-7 | 29 | 2.79 | | | | | | | | 0.05 | 0.22 |
| 1-9 | 31 | | | 4.7 | | | | | | | |
| 1-10 | 32 | | 0 | | | | | | | | |
| 1-30 | 52 | | 0 | 9.0 | 60 | 6.63 | 2.85 | 3.78 | 0.75 | | |
| 2-9 | 62 | | 0 | 9.5 | 100 | 5.4 | 2.6 | 2.8 | 0.93 | | |
| 2-21 | 74 | | | | | 6.45 | 3.2 | 3.2 | 1.0 | | |
| 3-15 | 96 | | 0 | 7.0 | | 6.39 | 2.5 | 3.89 | 0.64 | | |
| 3-28 | 109 | | | | | 5.73 | 3.0 | 2.73 | 1.10 | | |
| 4-7 | 119 | | 2+ | 8.0 | 100 | 6.18 | 3.3 | 2.8 | 1.18 | | |
| 4-19 | 130 | | | | | 6.97 | | | | | |
| 4-24 | 135 | | | | | 5.36 | 3.58 | 1.78 | 2.05 | | |

rum protein. Even at 96 days following the burn total serum proteins were still abnormal. The same changes were observed in two other patients, both of whom had adequate dietary intake.

In six patients who had burns of greater than 20 per cent third degree the changes in the liver function were seemingly much more marked. All patients had a marked retention of the B.S.P. dye. In all except two the cephalin flocculation was three plus or greater and there was a marked increase in the urine urobilinogen in all except one (O. B.) who developed severe oliguria and uremia. Four patients developed jaundice and five showed significant increases in the one minute fraction of the bilirubin determination. One man in this group was followed for a period of 32 days before his death and he developed outstanding changes in the total protein and albumin-globulin ratio. The albumin fraction remained for long periods around 1 Gm. and the total protein slightly over 5 Gm. per cent. These data are presented in Table IV. Electrophoretic studies of the serum proteins were performed on many of the patients reported in this paper. Changes were evident in all the protein fractions. This work is to be reported elsewhere (19).

In all third degree burns from the small to the most extensive there appears to be involvement of the functional state of the liver as judged by the usual liver function tests. It is suggested by our data that the more extensive the burn the greater the functional damage.

All of our patients who had more than the 20 per cent third degree burn succumbed and autopsies were performed on five of the six. The autopsy material demonstrated pathologic changes in the

TABLE IV

*F. T., W. M., 32, 30 per cent third degree burn*

| Date | Days post burn | Total protein | Albumin | Globulin | A/G ratio |
|---|---|---|---|---|---|
| | | *Gm. per cent* | *Gm. per cent* | *Gm. per cent* | |
| 9-22 | 0 | | | | |
| 9-23 | 1 | 4.8 | 3.2 | 1.6 | 2.0 |
| 9-24 | 2 | 4.7 | 2.9 | 1.8 | 1.61 |
| 9-25 | 3 | 3.6 | | | |
| 9-26 | 4 | 5.0 | | | |
| 9-27 | 5 | 4.3 | 2.4 | 1.9 | 1.26 |
| 1-4 | 12 | 5.1 | 1.3 | 3.8 | 0.33 |
| 1-7 | 15 | 5.1 | 1.1 | 4.0 | 0.37 |
| 1-11 | 19 | 5.2 | 1.0 | 4.5 | 0.22 |
| 1-14 | 21 | 5.5 | 1.7 | 3.8 | 0.44 |
| 1-15 | 22 | 4.9 | 1.7 | 3.8 | 0.44 |
| 1-18 | 25 | 6.2 | 2.9 | 3.3 | 0.88 |
| 1-28 | 35 | 4.8 | 2.4 | 2.4 | 1.00 |

livers of all the patients who died. There did not appear to be any definite pattern of pathologic alteration. In one man, F. T., with 30 per cent third degree and 95 per cent total burn who lived for 32 days the histology of the liver showed central vein changes, with sinusoidal congestion and generalized cloudy swelling and fat deposition more marked in the peripheral zones of the liver lobule. Many of the von Kupfer cells contained yellowish pigment, slightly granular in quality, which might be iron. These changes may be seen in Figures 1 and 1a. In another, O. B., a 29 year old negro female with 65 per cent third degree, there was a marked central congestion, degeneration, and necrosis, with an accumulation of polymorphonuclear neutrophiles in this area. As in F. T., yellowish brown pigment was prominent in the von Kupfer cells. In the areas of necrosis were observed macrophages with eosinophilic cytoplasm, and in the midzonal regions in addition to polymorphonuclear infiltration there were accumulations of lymphocytes with macrophages which appeared to be erythrophagocytic. Figures 2 and 2a show these changes. In another patient, G. L., a 51 per cent third degree, there were changes showing sinusoidal congestion, cloudy swelling, and minimal fatty degeneration in the central vein area. There appeared to be some increase in lymphocytes in the portal area. No focal necrosis was seen. The von Kupfer cells in some places appeared prominent. Figures 3 and 3a show the pathological changes.

That these changes occur early is indicated by the last two cases described. Both patients had severe burns and lived five and four days respectively. The necropsies were done within five hours post-mortem. The possibility that the changes observed were due to post-mortem autolysis unlikely.

The weights and gross appearance of the liv is recorded in Table V. Generally there was n much to be observed from the gross specimen other than slight enlargement. Gross fatty chang were not prominent.

It would appear from these observations that i addition to changes in liver function tests, the are histopathological changes in the liver. N correlation exists, and we have no biopsy da on the less severe burns. The mechanism by whic these changes were produced is not evident, but speculation leads us to wonder about the absorption of products of tissue disintegration from the burned surface. Shock was prevented and we have no reason to believe that there was a diminution of blood flow to the liver to produce these changes. Hourly urine output was good and was used as an index of renal blood flow. Certainly the greater the amount of third degree burn the more marked were the changes in liver function. We would tend to agree with the statements of Gillman and Gillman (5), that if the liver plays a part in causing the death of the burned individual it does so at a level where fundamental biochemical processes involving protein synthesis, detoxification, and probably reticulo-endothelial activity are impaired. The finding of erythrophagocytosis in one patient suggests that mechanism may play a part in the anemia of thermal burns.

The early appearance of changes in liver function suggests the advisability of initiating immediate preventive therapy against liver damage

TABLE V

| Patient | Amount of third degree | Hours post-mortem | Weight | Description |
|---|---|---|---|---|
| | *per cent* | | | |
| F. T.<br>W. M., 30 | 30 | 12 | 2,350 | External surface smooth, edges rounded, reddish-brown in color. Cut surface bleeds easily. |
| J. M.<br>N. M., 84 | 38 | 29 | 1,800 | Firm, smooth, light brown in color. Few pale areas in parenchyma. |
| G. L.<br>N. F., 19 | 51 | 5 | 1,870 | Bluish red, with firm consistency; internal architecture and vascular markings prominent. |
| F. J.<br>N. F., 20 | 55 | 15 | 1,425 | Surface pale yellowish and smooth; cut parenchymal surface bulges; lobular pattern distinct and generally yellowish color. |
| O. B.<br>N. F., 29 | 65 | 2 | 1,840 | Cut surface is fatty, bulges; congestion in the center of the lobule. |

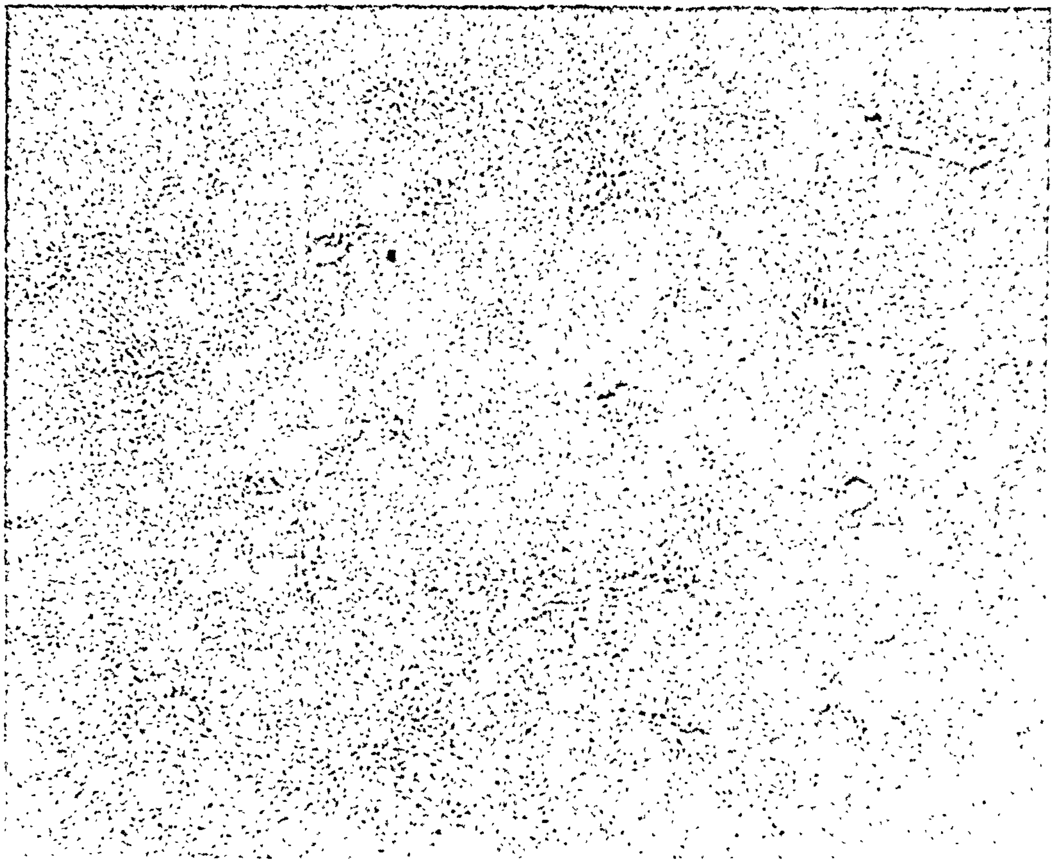

FIG. 1

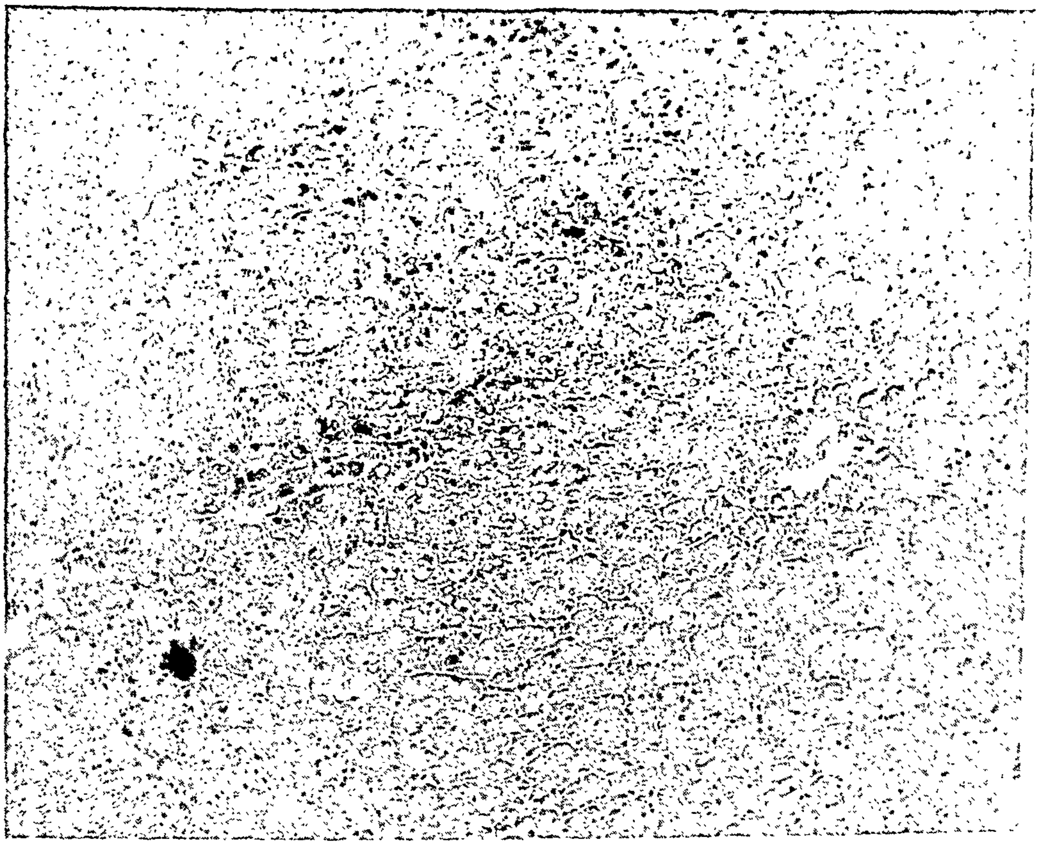

FIG. 1a

Figures 1 and 1a are taken of liver sections from patient F. T. who had 30 per cent third degree and 95 per cent total burn. Note the generalized cloudy swelling, sinusoidal congestion, and the fatty *infiltration and fat lobules in the* portal areas. There was marked urine urobilinogen excretion just prior to death.

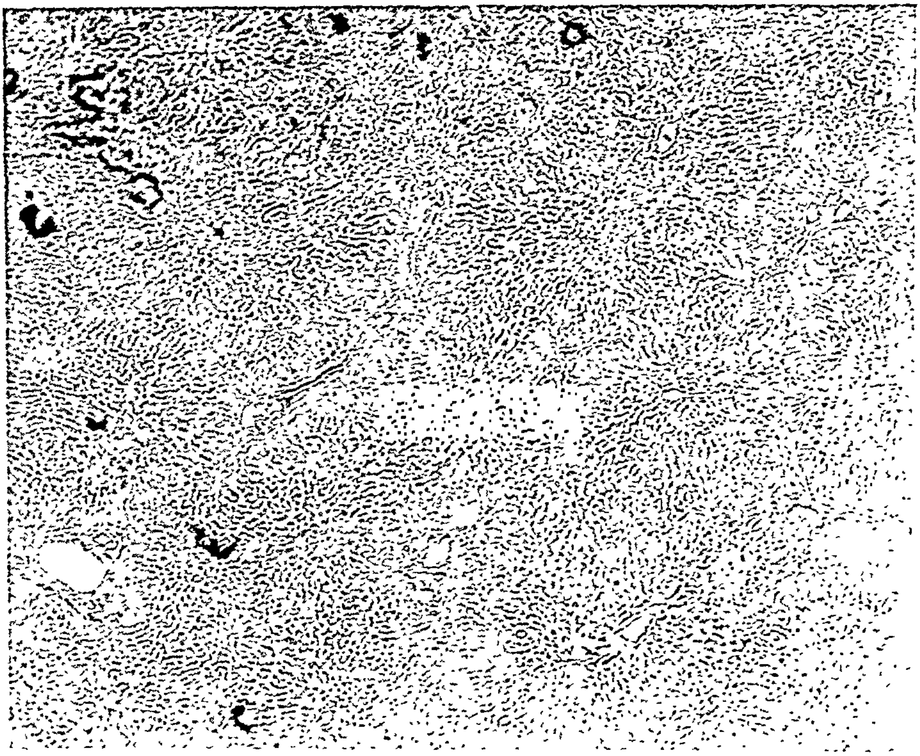

FIG. 2

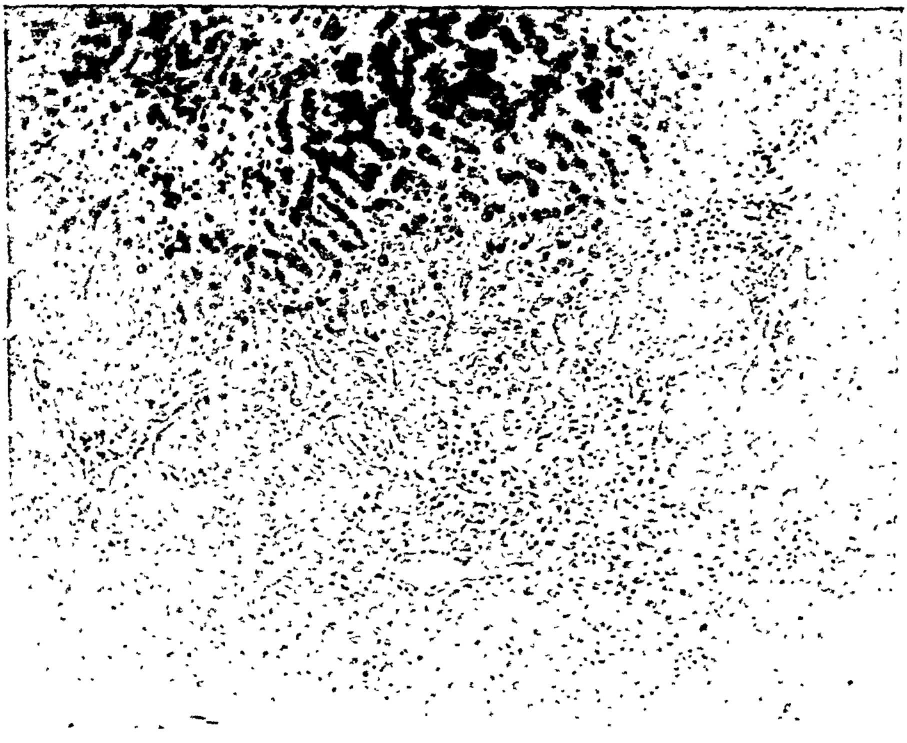

FIG. 2a

Figures 2 and 2a are taken of the liver sections from the patient O. B. with 65 per cent third degree burns. Note the marked sinusoidal congestion, areas of focal necrosis, and cloudy swelling. Eosinophilic macrophages were present and erythrophagocytosis observed. In some areas hyalinized eosinophilic bodies were seen.

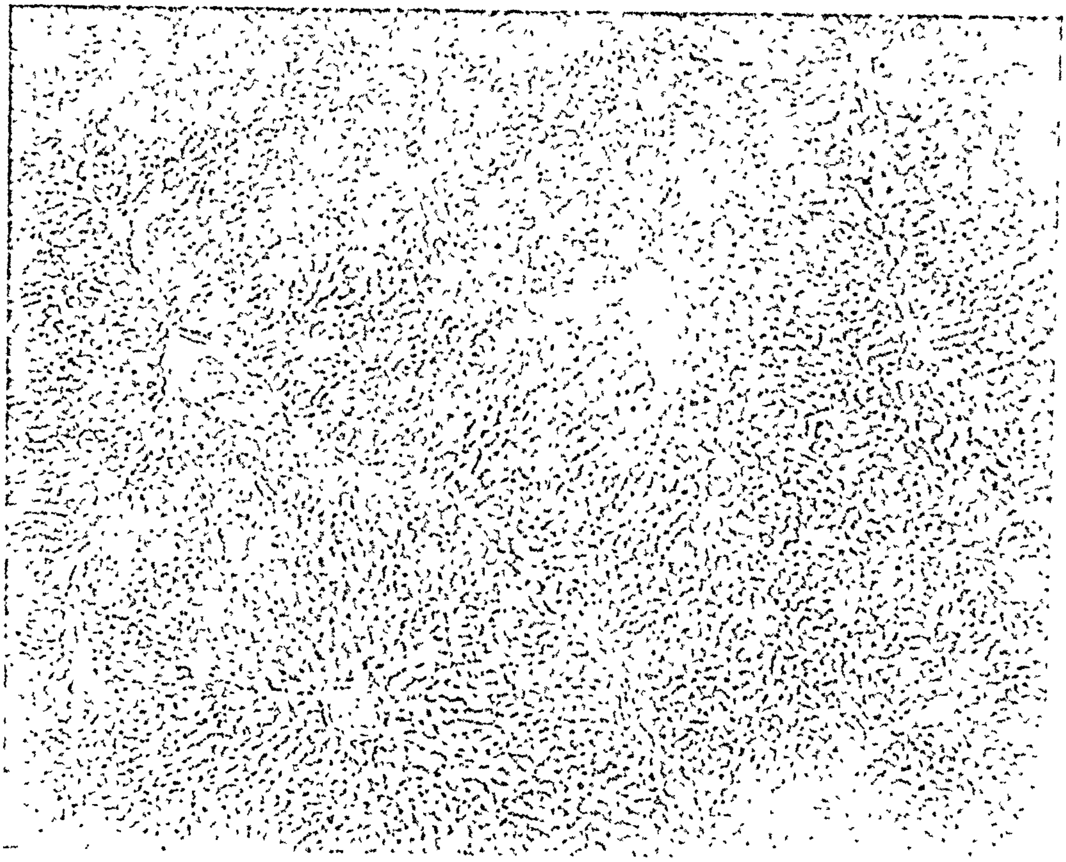

FIG. 3

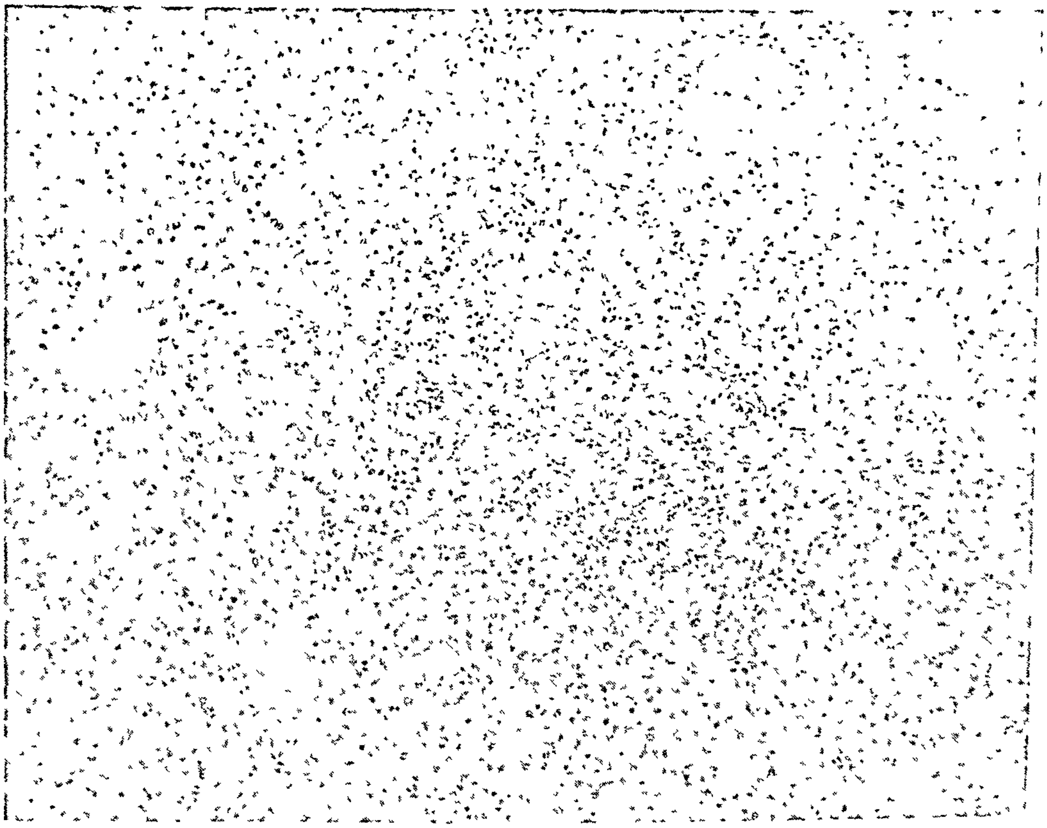

FIG. 3a

Figures 3 and 3a are from the liver sections of G. L. who suffered a 51 per cent third degree burn. There is sinusoidal congestion, cloudy swelling, and minimal fatty degeneration in the central vein areas. No focal necrosis was observed.

Two of the patients were on diets which provided very high protein and carbohydrate intakes with practically no fat. Milk protein and milk products formed the base of the diets and vitamin supplements were given in the quantities recommended by the National Research Council. The patients on this regime still manifested marked changes in the albumin-globulin ratio and in the B.S.P. retention as well as in the other liver function tests employed. We might speculate that this apparent disturbance in protein synthesis and/or excessive albumin loss probably has some part in the anemia of thermal injury through impaired globin synthesis.

## CONCLUSIONS

Minor to extensive third degree burns show early impairment of the liver function as judged by changes in the liver function tests employed in this study. The more severe the burn the greater the amount of change in liver function.

One of the most constant changes was the increase in urine urobilinogen excretion and alterations in the albumin-globulin ratio, both considered to be sensitive evidences of liver function.

Autopsy material on five fatal burns showed no constant histopathological change, but there was evidence of fatty infiltration, cloudy swelling, increased pigments in the reticulo-endothelial cells, focal necrosis, and congestion in the liver substance.

More study is necessary to understand the mechanism whereby liver function failure occurs and attention should be directed at the prevention, as well as treatment, of liver dysfunction in severe burns.

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# EFFECT OF DESOXYCORTICOSTERONE GLUCOSIDE UPON CEREBRAL BLOOD FLOW AND METABOLISM OF HUMAN SUBJECTS[1]

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(Submitted for publication August 8, 1950; accepted, November 13, 1950)

## INTRODUCTION

An effect of steroidal hormones upon cerebral metabolism was suggested by Selye's (1) observation that steroids possess anesthetic properties when administered to experimental animals. Gordan and Elliott (2) subsequently showed that certain steroids inhibit glucose utilization by rat brain *in vitro* and that their comparative inhibitory activity parallels their reported anesthetic potency. These observations were confirmed by Hayano, Schiller, and Dorfman (3). The possibility that steroidal inhibition of cerebral oxidative processes may be a physiological occurrence was demonstrated by Eisenberg, Gordan, and Elliott (4, 5). They found that deprivation of gonadal steroids by castration produces an increase in the rate of glucose utilization by rat brain and that the *in vivo* administration of testosterone, as well as other steroids with the androstene nucleus, prevents the characteristic rise. Desoxycorticosterone was the most potent of the steroidal compounds tested.

Evidence of an *in vivo* action of steroidal hormones on cerebral functions in man may be inferred from the observations of Pincus and Hoagland (6–9). In controlled studies, they found that the administration of pregnenolone to human subjects results in a significant lowering of fatigue responses to psychomotor tests. It is of interest that euphoria, depression, and exhilaration have been reported from the administration of various steroids in man (8–13).

The known anticonvulsive activity of desoxycorticosterone further suggests a steroidal influence on cerebral processes. Woodbury and Davenport (14) have demonstrated that the lowered electro-shock seizure threshold of adrenalectomized animals may be restored to normal levels, and that it may be increased above normal in intact animals by desoxycorticosterone acetate. In human epileptic patients, the anticonvulsive activity of this steroid has also been demonstrated. McQuarrie, Anderson, and Ziegler (15) and Aird and Gordan (16) have reported a reduction in the frequency of convulsive episodes of epileptic patients receiving desoxycorticosterone acetate. Desoxycorticosterone glucoside, an active water-soluble preparation of desoxycorticosterone, has been shown to reduce the electro-encephalographic abnormalities of epileptic patients (16).

These considerations have stimulated further investigation of steroidal activity upon cerebral metabolism in man. There is a suitable method, the nitrous oxide technique of Kety and Schmidt (17, 18), for the determination of cerebral blood flow in man. An estimation of the rate of cerebral blood flow permits a computation of the rate of utilization or production by the brain of any substance which can be accurately analyzed in blood. A steroidal compound well adapted for use in short-term studies in man is also available in the potent, water-soluble desoxycorticosterone glucoside (DCG).[3] The conjunction of a workable method for the estimation of the rate of cerebral blood flow and an active, water-soluble steroid has made feasible a measurement of steroidal effects on the metabolism of the human brain *in vivo*.

## METHOD

Cerebral blood flow (CBF) and cerebral metabolic rate (CMR) were estimated in 15 human subjects by the original nitrous oxide method of Kety and Schmidt (17,

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[1] Aided by a cooperative grant between the National Heart Institute and the University of California and a grant-in-aid from the Research Committee of the University of California.

[2] Schering Fellow in Endocrinology, 1949–1950.

[3] The desoxycorticosterone *glucoside* used in this study was generously furnished by Ciba Pharmaceutical Products, Inc., Summit, New Jersey.

18). Of the patients studied, seven had no endocrine disease, one had mild diabetes mellitus controlled by diet alone, two had been castrated for prostatic carcinoma, one represented Klinefelter's syndrome with hypoleydigism, and four had chromophobe adenomas with secondary hypogonadism. The subjects received no medications for 24 hours or more before the study and hormonal medications had been withheld for the preceding two or more weeks.

With the fasting subject supine, needles were inserted into the jugular bulb and either the femoral or internal carotid artery and connected to heparinized manifolds as described by Kety and Schmidt. In order to avoid the effects of emotional stimuli, as stressed by Scheinberg and Stead (19), no further procedures were undertaken until a steady state was achieved as determined by stable cardiac rate and blood pressure. In 14 of the 15 patients, two determinations of the rate of cerebral blood flow were made consecutively. Twenty minutes were allowed to elapse after the control determination to permit expiration of nitrous oxide, and a second blood blank for nitrous oxide was obtained in each case. Prior to proceeding with the second cerebral blood flow determination, specimens for control glucose levels of arterial, cerebral venous and, in seven cases, peripheral (antecubital) venous blood were obtained. All of these were drawn simultaneously at two-minute intervals until three separate sets of control specimens had been obtained.

Immediately after the third set of control samples for glucose determination were taken, 50 mgm. of DCG were injected intravenously and a second determination of the cerebral blood flow was made without delay. Blood sugar values were determined on aliquots of the simultaneously drawn experimental specimens of arterial and cerebral venous blood throughout the procedure for comparison with the previously drawn control specimens. Following completion of the second cerebral blood flow determination, two additional sets of specimens were obtained for analysis of sugar levels at three-minute intervals. When peripheral (antecubital) venous specimens were obtained, they were drawn simultaneously with the arterial and cerebral venous specimens. During each cerebral blood flow determination, arterial and cerebral venous oxygen and carbon dioxide content was determined upon separate specimens consisting of 5 cc. of blood drawn immediately before the first CBF sample and an equal amount drawn into the same syringe directly following the five-minute CBF specimen.

Blood oxygen and carbon dioxide analyses were made in duplicate by the manometric technique of Van Slyke and Neill (20), as modified by Kety and Schmidt (18). Blood sugar content was determined by the iodometric titration method of Somogyi (21, 22) and yielded results reproducible within 1 per cent. In accordance with the injunction of Somogyi (23) that, when dealing with changes in arterio-venous glucose difference, accurate analytical methods must be selected and employed with meticulous care, all glucose determinations were performed in duplicate by one of us (R.C.B.). Mean arterial blood pressure was obtained directly from the femoral or carotid artery by means of an air-damped mercury manometer connected to the arterial manifold. Cerebral oxygen consumption, cerebral glucose consumption or production, and cerebral carbon dioxide production were calculated from the rate of cerebral blood flow by the formulae of Kety and Schmidt.

## EXPERIMENTAL DATA

Experimental data are presented in Table I. For each function measured, the mean value and the standard deviation of the mean is included.

Striking and rapid effects were noted upon blood sugar levels following administration of 50 mgm. of DCG. Uniformly, the cerebral venous glucose concentration rose significantly—in eight instances above the arterial level—effecting in 13 of the 15 cases a reduction in the cerebral arterio-venous glucose difference. While the individual changes in the arterial and peripheral (antecubital) venous blood sugar concentrations were inconsistent and not marked, a small, but statistically significant ($p < 0.01$), rise occurred in both the mean arterial and the mean antecubital venous blood sugar concentrations following the administration of DCG. Examples of the glucose concentration curves are shown in Figures 1 and 2. It should be noted that the changes in glucose concentration occurred without alteration in the rate of cerebral blood flow. Figure 3 depicts the change in mean cerebral arterio-venous glucose difference produced by DCG. This demonstrates

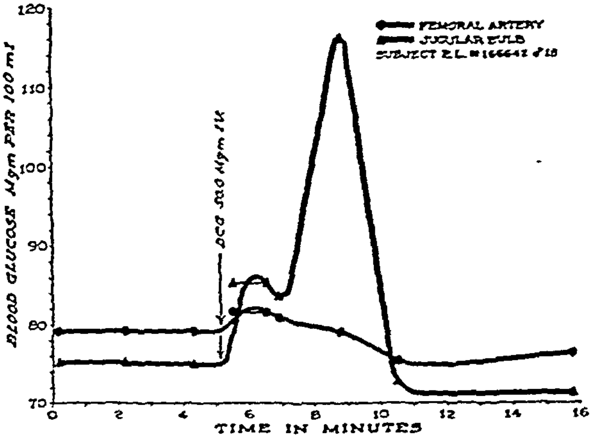

FIG. 1. EFFECT OF DCG ON CEREBRAL VENOUS AND ARTERIAL GLUCOSE CONCENTRATIONS

Note that the cerebral venous sugar level rises rapidly above the arterial glucose level and remains elevated for approximately five minutes.

TABLE I

*The effect of desoxycorticosterone glucoside* (DCG) *on cerebral blood flow and metabolism*

| No. | Subject | Sex | Age | Diagnosis | CBF* cc./100 gm./min. | | MABP† mm. Hg. | | Cardiac rate per minute | | A-V$_{oxygen}$ Vol. per cent | | CMR$_{oxygen}$ cc./100 gm./min. | | A-V$_{CO_2}$ Vol. per cent | | CMR$_{CO_2}$ cc./100 gm./min. | | Maximum A-CV‡ glucose‖ mgm. per cent | | CMR$_{glucose}$ mgm./100 gm./min. | | Maximum A-PV§ glucose‖ mgm. per cent | | Maximum change in glucose after DCG¶ mgm. per cent | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Control | DCG | Control | DCG | Control | DCG | Control | DCG | Control | DCG | Control | DCG | Control | DCG | Control | DCG | Control | DCG | Control | DCG | CV | Arterial | PV |
| 1 | M. C. | F | 35 | No endocrine disease | 73 | 33 | 78 | 72 | 76 | 76 | 4.5 | 4.4 | 3.3 | 1.5 | 3.6 | 2.9 | 3.4 | 1.0 | + 4 | −19 | + 3 | − 6 | + 5 | + 1 | ↑ 26 | ↓ 2 | ↑ 5 |
| 2 | H. R. | M | 17 | No endocrine disease | 37 | 27 | 68 | 68 | 75 | | 5.0 | 6.4 | 1.9 | 1.7 | 3.4 | 3.2 | 1.3 | 0.9 | + 4 | − 5 | + 1 | − 1 | | | ↑ 11 | ↑ 7 | |
| 3 | D. A. | F | 46 | No endocrine disease | 52 | 36 | 105 | 105 | 94 | 90 | 7.5 | 7.8 | 3.9 | 2.8 | 7.0 | 7.2 | 3.6 | 2.6 | + 9 | − 3 | + 4 | − 1 | | | ↑ 8 | ↓ 4 | |
| 4 | D. P. | M | 48 | No endocrine disease | 47 | 36 | 105 | 105 | 60 | 60 | 7.8 | 7.6 | 3.7 | 2.7 | 7.6 | 5.8 | 3.6 | 2.1 | +13 | − 9 | + 6 | − 3 | +12 | +10 | ↑ 22 | 0 | ↑ 2 |
| 5 | W. W. | M | 29 | No endocrine disease | 109 | 86 | 58 | 58 | 64 | 64 | 5.4 | 5.7 | 5.9 | 4.9 | 3.8 | 3.7 | 3.4 | 3.2 | + 7 | + 3 | + 8 | + 3 | +16 | +14 | ↑ 10 | ↑ 6 | ↑ 6 |
| 6 | F. N. | M | 44 | No endocrine disease | 56 | 75 | 158 | 162 | 82 | 80 | 4.7 | 5.0 | 2.6 | 3.8 | 3.3 | 5.1 | 1.9 | 3.8 | + 6 | + 1 | + 3 | + 1 | + 6 | + 6 | ↑ 3 | ↑ 8 | ↑ 2 |
| 7 | H. H. | M | 53 | Diabetes mellitus | 38 | 32 | 60 | 60 | 84 | 84 | 9.4 | 6.5 | 3.6 | 2.1 | 9.3 | 7.0 | 3.5 | 2.2 | +26 | +17 | +10 | + 5 | | | ↑ 3 | ↓ 6 | |
| 8 | L. D. | M | 34 | Chromophobe adenoma with secondary hypogonadism | 60 | 78 | 82 | 86 | 70 | 72 | 6.4 | 5.6 | 3.8 | 4.4 | 6.1 | 5.9 | 3.7 | 4.7 | + 6 | − 2 | + 3 | − 1 | | | ↑ 4 | ↓ 4 | |
| 9 | M. R. | F | 51 | Chromophobe adenoma | 53 | | 90 | 90 | 80 | 74 | 2.0 | 3.6 | 1.1 | | 2.9 | 4.8 | 1.5 | | + 3 | + 2 | + 2 | | | | ↑ 1 | ↑ 3 | |
| 10 | L. L. | M | 35 | Chromophobe adenoma | | | | | | | 6.6 | 6.9 | | | 5.7 | 5.6 | | | + 4 | + 4 | | | + 1 | + 4 | ↑ 5 | ↑ 5 | ↑ 2 |
| 11 | E. L. | M | 18 | Chromophobe adenoma with secondary hypogonadism | 57 | 65 | 65 | 68 | 80 | 80 | 5.1 | 3.5 | 2.9 | 2.3 | 5.6 | 6.5 | 3.2 | 4.2 | + 4 | −38 | + 2 | −25 | | | ↑ 42 | ↓ 1 | |
| 12 | R. W. | M | 22 | Klinefelter's syndrome with hypoleydigism | 71 | 83 | 78 | | 80 | | 4.8 | 3.7 | 3.4 | 3.1 | 3.3 | 3.4 | 3.1 | 2.9 | + 6 | − 9 | + 4 | − 8 | | | ↑ 24 | ↑ 9 | |
| 13 | W. P. | M | 49 | Primary testicular eunuchoidism | 48 | 41 | 65 | 69 | 66 | 68 | 5.3 | 4.8 | 2.5 | 2.0 | 3.6 | 2.8 | 1.7 | 1.2 | + 3 | + 5 | + 1 | + 2 | + 5 | +10 | ↑ 6 | ↑ 8 | 0 |
| 14 | J. B. | M | 69 | Castrate (carcinoma of prostate) | 62 | 61 | 98 | 98 | 62 | 62 | 3.3 | 5.9 | 2.0 | 4.2 | 3.8 | 7.0 | 2.4 | 4.3 | + 3 | − 6 | + 2 | − 3 | | | ↑ 19 | ↑ 10 | |
| 15 | A. H. | M | 64 | Castrate (carcinoma of prostate) | 55 | 57 | 150 | 135 | 83 | 80 | 4.6 | 4.9 | 2.5 | 2.8 | 3.9 | 5.2 | 2.1 | 2.9 | +12 | + 9 | + 7 | + 5 | + 4 | + 4 | ↑ 10 | ↑ 7 | 0 |
| | Mean of all observations.........<br>Standard deviation of the mean......... | | | | 58<br>± 5 | 55<br>± 6 | 90<br>± 9 | 91<br>± 9 | 75<br>± 3 | 74<br>± 3 | 5.5<br>±0.5 | 5.5<br>±0.4 | 3.1<br>±0.3 | 2.8<br>±0.3 | 4.9<br>±0.5 | 5.1<br>±0.4 | 2.7<br>±0.2 | 2.8<br>±0.4 | + 7.2<br>± 1.6 | − 3.3<br>± 3.5 | + 4.1<br>± 0.8 | − 2.6<br>± 1.3 | + 6.8<br>± 2.0 | + 6.9<br>± 1.8 | ↑ 13.3<br>± 3.0 | ↑ 3.1<br>± 1.3 | ↑ 3.9<br>±0.8 |

* CBF = Cerebral blood flow in cc./100 gm. of brain/minute.

† MABP = Mean arterial blood pressure.

‡ CV = Cerebral venous blood from internal jugular bulb.

§ PV = Peripheral venous blood from antecubital vein.

‖ Positive figure indicates arterial concentration greater than venous; negative figure indicates venous concentration greater than arterial.

¶ ↑ indicates increase after DCG.
↓ indicates decrease after DCG.

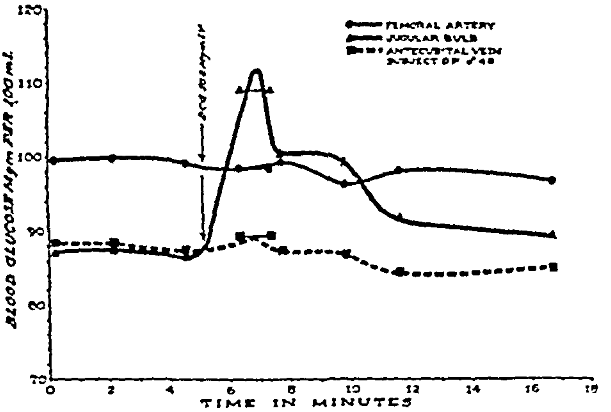

FIG. 2. EFFECT OF DCG ON SUGAR CONTENT OF ARTERIAL AND ON CEREBRAL AND PERIPHERAL VENOUS BLOOD

Note prompt rise of cerebral venous sugar level above arterial glucose concentration in contrast to stable peripheral venous glucose level.

an increase of the mean cerebral venous glucose concentration above the mean arterial glucose concentration after administration of DCG, and, in conjunction with the statistically significant change in $\mathrm{CMR}_{\mathrm{glucose}}$, is interpreted to indicate liberation of glucose from the brain.[4]

The mean rates of cerebral blood flow, oxygen consumption, and carbon dioxide production did not change significantly. As previously noted by Green and his associates (24), DCG did not alter the mean arterial blood pressure or cardiac rate. No immediate or subsequent clinical effects were apparent.

## DISCUSSION

These experimental findings lend the support of quantitative measurements in man to the thesis that steroidal hormones may affect cerebral carbohydrate metabolism. The consistently observed increase in cerebral venous glucose concentration, with a decrease in the cerebral arterio-venous glucose difference produced by DCG in the absence of increased cerebral blood flow, could have resulted from either diminished cerebral utilization of glucose, liberation of sugar by the brain, or both. An increase in the sugar concentration of the cerebral venous blood above that of arterial blood obtained simultaneously indicates liberation of sugar by the brain. From these observations alone, however, it is not possible to conclude that there is not also an alteration of the cerebral utilization of glucose.

From the present data, the mechanism and specificity of this effect may be only conjectured. Of the several possible sources for the sugar liberated from the brain following DCG, a derivation from intracellular glycogen stores may be considered. Kerr and Ghantus (25) have demonstrated sufficient quantities of glycogen in the brains of dogs and rabbits to account for the amount of sugar "liberated" following injection of DCG. The presence of some endogenous substrate in human brain which can be utilized for cerebral oxidative processes *in vitro* is suggested by Elliott, Sutherland, and Boldrey (26). They found that the oxygen consumption of slices of human cerebral cortex was maintained for a significantly longer period in the absence of a glucose substrate than was the oxygen consumption of rat brain, which dropped off rapidly. If this substrate is glycogen, the glycogenolytic potentiality of desoxycorticosterone reported by Verzar and Wenner (27) would be in harmony with our observations.

Other sources must also be considered, among them the intracellular and extracellular glucose

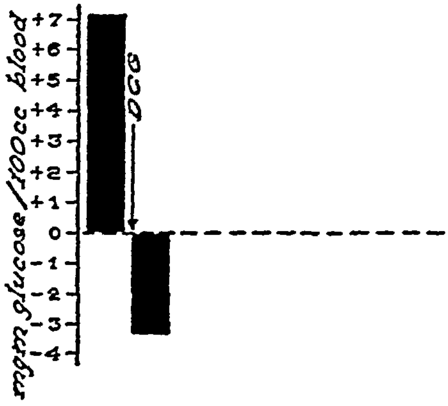

FIG. 3. CEREBRAL ARTERIO-VENOUS GLUCOSE DIFFERENCE: MEAN VALUES FOR 15 SUBJECTS BEFORE DCG AND AT THE PEAK RESPONSE AFTER DCG

Positive value before DCG indicates that the arterial glucose concentration is higher than the cerebral venous glucose level (glucose utilization). Negative value after DCG indicates that the cerebral venous glucose level exceeds the arterial concentration (glucose "liberation").

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[4] As performed, the Somogyi method is a measure of reducing substance content, almost exclusively monosaccharides. Subsequent to the preparation of this report, we have found that the bulk of the reducing substance liberated into the cerebral venous blood is non-fermentable, and preliminary attempts at identification suggest galactose.

and cerebral galactosides, but data regarding these are not yet available. Further studies to extend these observations and their specificity are in progress.

No significant alteration in the mean $CMR_{O_2}$ or $CMR_{CO_2}$ was observed, indicating that desoxycorticosterone glucoside does not influence the aerobic phase of cerebral energy metabolism as an immediate general effect, although it may alter the substrate. However, it is interesting that the cerebral metabolic rate was diminished following administration of DCG in all but one ortho-steroidal subject and that no change or only a slight increase in cerebral metabolic rate was apparent in all hyposteroidal subjects. Until the individual groups are expanded, it is not possible to establish the significance of this observation. In this connection it is recognized that the data included in the present paper group together observations made upon individuals with divergent endocrine states. The data suggest that enlargement of the groups may eliminate some of the variability due to the lumping together of non-comparable individuals. The fact that the standard deviations of the mean in this study are somewhat greater than those reported by other workers may, in part, be explained on this basis.

While the arterio-venous sugar difference reflected in the cerebral circulation is altered, no such change occurs in the peripheral arteriovenous glucose difference, suggesting the absence of an immediate effect of desoxycorticosterone glucoside upon the glucose metabolism of the muscular tissues of the forearm. The small but significant rise in the mean arterial glucose concentration following administration of DCG might be accounted for by liberation of glucose from other sources, possibly the liver.

## SUMMARY

1. The effects of desoxycorticosterone glucoside upon the cerebral utilization of oxygen and glucose and the production of carbon dioxide in human subjects has been studied by the nitrous oxide technique of Kety and Schmidt.

2. Administration of DCG produced a rise in the cerebral venous sugar concentration above the arterial level, indicating that this compound causes liberation of sugar from the brain.

3. A small, but significant, increase in arterial glucose concentration also occurred, suggesting liberation of glucose from other sources, possibly the liver.

4. Preliminary data suggest a different response to DCG between individuals with normal and deficient steroidal status.

5. The mean arterial blood pressure, cardiac rate, and rate of cerebral blood flow were not altered by DCG.

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# THE RELATION BETWEEN PROTEIN CONSUMPTION AND DIURNAL VARIATIONS OF THE ENDOGENOUS CREATININE CLEARANCE IN NORMAL INDIVIDUALS[1]

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(Submitted for publication September 11, 1950; accepted, November 13, 1950)

In a previous paper based on the same study (1), it was shown that the serum urea concentration of subjects with intact renal function increases with protein consumption. In contrast, the serum creatinine concentration in such subjects was shown to be independent of protein consumption (2). It has long been known that endogenous urea excretion increases with the protein consumed, while the rate of endogenous creatinine excretion has been shown to be relatively independent of the amount of protein consumed (3).

Popper and Mandel (4) first utilized the endogenous creatinine clearance in man as a measure of the glomerular filtration rate. Steinitz and Türkand (5) found a good correspondence between the inulin and endogenous creatinine clearances in normal subjects. More recently Brod and Sirota (6) have studied the endogenous creatinine chromogen/inulin clearance ratio in man, and concluded that the endogenous creatinine clearance corresponds closely to the inulin clearance in normal subjects. Discrepancies which appear in subjects with renal disease and markedly impaired renal function, are not sufficient to affect seriously the clinical value of the test as a general indicator of renal competence. This judgment has been confirmed by Camara (7), as well as by numerous clinical observations of our own.

Miller and Dubos (8) showed that creatinine is not the only chromogen in human serum that produces color in the Jaffé reaction. The ratio of such "pseudocreatinine" to creatinine is higher in azotemic individuals. Brod and Kotátko (9) found that the endogenous creatinine and the inulin clearances approach identity only when the "true" creatinine clearance is used. They used Lloyd's reagent to adsorb creatinine and determined the residual "pseudocreatinine" concentration in the serum.

Diurnal variations in the endogenous creatinine clearance have been studied by Brod (10). He found that the endogenous creatinine clearance in normal individuals is depressed during the night. In a more complete investigation, Sirota, Baldwin and Villarreal (11) studied diurnal variations in renal function of normal male subjects. They found no significant difference in the night and day values for the endogenous creatinine clearance, except for a slight fall in the period from 12 Midnight to 4 A.M. These experiments were conducted on "routine ward" diets, and no attempt was made to vary or to control at a constant level the consumption of protein or of creatinine-containing foods.

Of all renal function tests, the endogenous creatinine clearance is easiest to perform since no foreign substance is infused, the serum concentration is constant, and urine collections can be made over long periods of time, up to 24 hours, thus minimizing the errors in collection. Those who have struggled with the methods for performing inulin determinations realize the relative precision and simplicity of the creatinine determination, as modified by Bonsnes and Taussky (12). For these reasons it seemed desirable to investigate the effect of dietary protein consumption upon the endogenous creatinine clearance and its diurnal variation, since this matter has not been considered in the previous publications.

A clinical test is of optimal usefulness if its performance is simple. For this reason, because we were concerned with the endogenous creatinine clearance as an indicator of renal competence without regard to the possible analysis of indi-

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[1] This work, performed in 1945–46, was made possible by a grant from the Nutrition Foundation, Inc.

[2] Dr. Addis died June 4, 1949.

[3] Fellow of the John Simon Guggenheim Memorial Foundation.

TABLE I

*Diurnal variation of* serum creatinine concentration *in relation to dietary protein consumption in normal male individuals*

| Diet | 7:15 A.M. | | | 11:45 A.M. | | | 4:45 P.M. | | | 9:45 P.M. | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| g. protein/kg. | no. obs. | mg. % | stand. dev. mean | no. obs. | mg. % | stand. dev. mean | no. obs. | mg. % | stand. dev. mean | no. obs. | mg. % | stand. dev. mean |
| 0.5 | 26 | 1.02 | ±0.08 | 34 | 1.03 | ±0.09 | 17 | 1.08 | ±0.11 | 17 | 1.06 | ±0.06 |
| 1.5 | 18 | 1.00 | ±0.09 | 18 | 1.01 | ±0.09 | 8 | 1.05 | ±0.07 | 9 | 1.09 | ±0.07 |
| 2.5 | 34 | 1.04 | ±0.07 | 36 | 1.04 | ±0.07 | 17 | 1.04 | ±0.07 | 17 | 0.99 | ±0.09 |

vidual renal functions, it was not considered desirable to complicate the determinations by the use of Lloyd's reagent. In addition, the test is most useful in evaluating patients with slight degrees of renal functional impairment, before the development of azotemia. In such patients the "pseudocreatinine" chromogen is at a minimum.

## METHOD

### 1. *Conditions of the experiment*

The conditions have been previously described in detail (1, 2). The principal subjects in this study were nine male internes. They were fed carefully contrived diets, all providing more than 2,000 calories per day, with fluid consumption permitted *ad libitum.* From Monday breakfast (7 A.M.) until Saturday at noon the diet was carefully followed. Over the weekend, until the following Monday at breakfast, the subjects were allowed a free diet. The breakfasts were free of pre-formed creatinine; that is, they contained no meat, fish, or chicken. At other meals meat was used as needed to provide the desired amount of protein. During the first week the diet contained 0.5 g. protein per kg. body weight per day. During the second week the protein content of the diet was 1.5 g. per kg. body weight, and during the third week the protein content was raised to 2.5 g. per kg. body weight. After a gap of two months, the experiment was repeated at lowest and highest levels of protein consumption.

Urine collections and blood specimens were obtained on the last two days of each diet. Friday was divided into four urine collection periods; 7 A.M. to 12 Noon, 12 Noon to 5 P.M., 5 P.M. to 10 P.M. and 10 P.M. to 7 A.M. Blood specimens were obtained just before each meal and at bedtime, with a 15 minute leeway allowed in either direction from the times indicated: 7:15 A.M., 11:45 A.M., 4:45 P.M. and 9:45 P.M. On Saturday there was a single urine collection from 7 A.M. to 12 Noon, while blood specimens were obtained at 7:15 A.M. and 11:45 A.M.

In order to compare the normal clearance values in women with those in men, a group of 25 female nurses, dietitians and medical students were studied in a similar fashion. However, the experiment was modified for this group and they were studied only on the 0.5 g. protein diet. Urine collections were made each day of the week from 10 P.M. to 7 A.M., while blood specimens were taken daily at 3:15 P.M.

### 2. *Methods of Measurement*

Creatinine determinations were performed by the Folin-Wu method, as modified (2). Clearances were calculated from the measured excretion rate, expressed in mg. per 24 hours, corrected to a body weight of 70 kg. The serum concentration used in the calculation was a logarithmic mean of the concentrations found at the beginning and the end of each period. For body weight corrections, the subjects were weighed daily. Clearance values were corrected by a factor $\frac{70^{0.7}}{BW^{0.7}}$, where BW = body weight in kg., in accordance with the suggestion of Addis (13). The calculated clearances were expressed in L. per 24 hours per 70 kg. body weight.

## RESULTS

As has previously been shown (2), the serum creatinine concentration does not vary significantly with diet in normal individuals. From Table I it is seen that the diurnal variation in serum cre-

TABLE II

*Diurnal variation of* endogenous creatinine excretion *in relation to dietary protein consumption in normal male individuals*

| Diet | 7 A.M.–12 Noon | | | 12 Noon–5 P.M. | | | 5 P.M.–10 P.M. | | | 10 P.M.–7 A.M. | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| g. protein/kg. | no. obs. | mg./24 hrs./70 kg. | stand. dev. mean | no. obs. | mg./24 hrs./70 kg. | stand. dev. mean | no. obs. | mg./24 hrs./70 kg. | stand. dev. mean | no. obs. | mg./24 hrs./70 kg. | stand. dev. mean |
| 0.5 | 31 | 1760 | ±164 | 16 | 1783 | ± 77 | 16 | 1823 | ±119 | 16 | 1517 | ±97 |
| 1.5 | 16 | 1858 | ±112 | 9 | 1896 | ± 81 | 9 | 1935 | ± 83 | 9 | 1611 | ±78 |
| 2.5 | 33 | 1844 | ±155 | 14 | 1988 | ±135 | 18 | 2081 | ±150 | 17 | 1687 | ±90 |

TABLE III

*Diurnal variation of* endogenous creatinine clearance *in relation to dietary protein consumption in normal male individuals*

| Diet | 7 A.M.–12 Noon | | | 12 Noon–5 P.M. | | | 5 P.M.–10 P.M. | | | 10 P.M.–7 A.M. | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| g. protein/kg. | no. obs. | L./24 hrs./70 kg. | stand. dev. mean | no. obs. | L./24 hrs./70 kg. | stand. dev. mean | no. obs. | L./24 hrs./70 kg. | stand. dev. mean | no. obs. | L./24 hrs./70 kg. | stand. dev. mean |
| 0.5 | 24 | 171 | ±17 | 16 | 167 | ±15 | 16 | 166 | ±15 | 16 | 141 | ±12 |
| 1.5 | 16 | 180 | ±17 | 8 | 177 | ±10 | 8 | 179 | ±14 | 9 | 151 | ±15 |
| 2.5 | 31 | 178 | ±17 | 14 | 182 | ±17 | 16 | 200 | ±14 | 16 | 161 | ±13 |

atinine concentration is insignificant, with a wide range of protein consumption, even though the consumption of pre-formed creatinine was limited to the noon and evening meals.

As seen in Table II, on each diet there is a moderate but significant drop in creatinine excretion during the period from 10 P.M. to 7 A.M. In addition, there is a slight but significant rise in creatinine excretion during all periods, except 7 A.M. to 12 Noon, as the dietary protein consumption rises. Since the serum creatinine concentration remains constant, the clearance values follow the variations in excretion, and this may be seen in Table III.

The female subjects were observed only under one set of conditions, so that effects of diet and diurnal variation were not noted. However, Table IV reveals that both the serum creatinine concentration and the creatinine excretion were lower than the values obtained in males on the same diet and at the same times. In spite of this the mean clearance value was not significantly different from the value obtained in males under similar conditions.

## DISCUSSION

Since there was a drop in creatinine excretion during the period from 10 P.M. to 7 A.M. and there was a rise during the period from 7 A.M. to 12 Noon, after a creatinine-free breakfast, on all diets, the factor responsible for the nocturnal drop must be other than the dietary protein consumption. In view of the observations of others that various renal functional measurements rise during recumbency and drop when the erect, *lordotic* posture is assumed (14–16), it seems improbable that the nocturnal drop in creatinine excretion is a postural effect. Likewise, it seems improbable that this nocturnal drop is the result of diminished physical activity, since several investigators have found that renal functional measurements made at rest are greater than those made during mild exercise and much greater than those made during severe exercise (17–19).

TABLE IV

*Nocturnal creatinine clearance values in normal females on a low protein diet (0.5 g. per kg. per day)*

| | No. obs. | Value | Stand. dev. mean |
|---|---|---|---|
| Serum creat. conc. (3 P.M.) | 124 | 0.79 mg. % | ±0.07 |
| Creat. excret. (10 P.M.–7 A.M.) | 124 | 1147 mg./24 hrs./70 kg. | ±94 |
| Creat. clear. (10 P.M.–7 A.M.) | 124 | 146 L./24 hrs./70 kg. | ±14 |

During sleep a much greater degree of relaxation is attained than during rest while awake. There is a generalized relaxation of the vascular bed, manifested by the drop in vascular tension that occurs during sleep in normal individuals. It seems most reasonable to suppose that the nocturnal drop in creatinine excretion, and consequently in the endogenous creatinine clearance, is the result of such a relaxation of the vascular tension, with a drop in filtration pressure at the glomerulus and drop in the glomerular filtration rate. In this connection it is of interest to note that Sirota and his associates (11), by using shorter nocturnal urine collection periods, found the period of diminished inulin and endogenous creatinine clearances to coincide with the period of deepest sleep.

The influence of diet upon the endogenous creatinine clearance is absent in the morning period from 7 A.M. to 12 Noon, after a creatinine-free breakfast. From this it is apparent that the fasting period from supper time until morning is sufficient to obtain basal conditions with respect to creatinine excretion. For this reason, it seems most desirable for clinical determinations to use the morning period for collection, after a creatinine-free breakfast. This simply means a normal breakfast of eggs, toast, cereal, etc., but without meat or bacon.

In this experiment, there was no significant variation in the serum creatinine concentration of the male subjects under any of the varied conditions, with a grand mean value of 1.03 mg. per 100 ml. Therefore, it would seem satisfactory to use a serum specimen obtained just before, during, or just after the period of urine collection for calculation of the clearance.

In the female subjects the serum creatinine concentration was slightly, but significantly, lower than in the males, with a mean value of 0.79 mg. per 100 ml. However, the creatinine excretion was proportionately lower than in the males, so that the mean endogenous creatinine clearance did not differ significantly from the value obtained in males under the same conditions.

The values obtained for the endogenous creatinine clearance during the morning collection period from 7 A.M. to 12 Noon had a mean value of 176 L. per 24 hours per 70 kg. body weight. This is almost identical with the values obtained by Sirota and his colleagues (11) for the "peak," when translated into comparable units.

## SUMMARY

The endogenous creatinine clearance is a valuable, simple, clinical test of renal function. It is affected slightly by the dietary protein consumption and by diurnal variations. These influences may be eliminated, for practical purposes, by collecting urine for the test during the morning after a creatinine-free breakfast. The normal endogenous creatinine clearance under such conditions is 176 L. per 24 hours per 70 kg. body weight, or 122 ml. per minute per 70 kg. body weight.

In females the normal mean serum creatinine concentration is 0.79 mg. %, slightly lower than in males. The normal mean endogenous creatinine clearance for female subjects was determined under one set of conditions and did not vary significantly from the values obtained in males.

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# THE CARDIAC OUTPUT IN PATIENTS WITH ARTERIOVENOUS FISTULAS[1]

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(Submitted for publication October 21, 1950; accepted, November 20, 1950)

The study of arteriovenous fistulas, and the alterations in local and systemic circulation which they produce, has been a means of not only understanding this particular lesion better, but of extending our knowledge of many other more common circulatory phenomena. In peacetime, investigations have been conducted on the relatively rare clinical cases and on animals with fistulas produced in the laboratory. The recent war, with its many vascular injuries, presented an unusual opportunity to study the circulatory alterations in patients with arteriovenous fistulas. This and succeeding reports deal with observations on a large group of patients treated at Ashford General Hospital.

The clinical manifestations exhibited by patients with an arteriovenous fistula have been described elsewhere (1, 2). It is generally recognized that these patients may develop congestive heart failure. There has been controversy, however, regarding the mechanism producing the circulatory failure. Some have ascribed it to greatly increased demands on the heart, others to diminution of coronary blood flow secondary to the lowered mean arterial pressure (3). Since a knowledge of the output of the heart is so important in defining the mechanism of the altered circulatory dynamics in these patients, we have measured the cardiac output as part of a general study of the circulation in patients with arteriovenous fistulas.

In 1923 Lewis and Drury (4) reported the results of their studies of arteriovenous fistulas in both man and the experimental animal. In the latter, using a glass cardiometer to enclose the heart, they found an increase in cardiac output, in some instances, when the fistula was open. In the animals with an elevated cardiac output they also noticed an increased central venous pressure. Because the venous pressure was found to be normal in their human cases, they assumed that the cardiac output was normal. No actual measurements on humans were reported. Harrison, Dock and Holman (5) in 1924, using the direct Fick technique, reported that the cardiac output was elevated in their animals with experimental arteriovenous fistulas. Since then, several additional studies in experimental animals have confirmed the earlier observations that the basal cardiac output may be elevated by the creation of an arteriovenous fistula (3, 6–8). On the other hand, the relatively few clinical observations made on patients with arteriovenous fistulas led to less definitive conclusions; in most instances an increased cardiac output was found (9–14). These data have been summarized in two recent reports (3, 14). It may be that the foreign gas methods, often used in McMichael clinical studies, were invalidated by the abnormal circulatory dynamics present in these patients. Insofar as we are aware, there is no study reported of the cardiac output in a large group of patients observed before and after removal of an arteriovenous fistula.

## METHODS

The cardiac output was determined by means of the low frequency, critically damped ballistocardiograph (15, 16) which measures the output by the *ballistic recoil* of the body with each heart beat.

To verify the validity of the measurements by the ballistocardiograph in the presence of arteriovenous fistulas or large areas of reactive hyperemia (in reality a temporary arteriovenous fistula) comparative studies by the direct Fick technique were made (16–19). A satisfactory correlation between the two methods was demonstrated. We believe, therefore, that, under the conditions of the present study, the ballistocardiographic method is a useful and valid one.

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[1] The work described in the paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the Emory University School of Medicine, Atlanta, Georgia.

To facilitate comparison of individuals of various sizes, the cardiac index (the output of the heart in liters per minute per square meter of body surface) was used in recording our observations. All determinations were made on subjects three hours or more after they had eaten, and after they had relaxed for at least 15 minutes on the ballistocardiograph table. The arterial pressure was obtained with an ordinary mercury manometer by the auscultatory method. In patients with a fistula sufficiently distal on an extremity so that occlusion could be accomplished by inflation of a blood pressure cuff, observations were made before and during the occlusion of the fistula. These studies are reported separately (20). The transverse diameter of the heart as measured from a teleoroentgenogram was used as an index of changes in heart size. The blood volume was measured by means of the blue dye T-1824 (21).

## RESULTS

Forty-seven patients with arteriovenous fistulas were studied at least once before and after operation. Many had a cardiac output determination on several occasions. All the patients were presumably normal prior to their injury resulting in the establishment of the arteriovenous fistula. All except on were men. The time between the injury causing the fistula and the operative repair varied from two months to slightly over two years. No patient had evidence of frank congestive heart failure. All the patients had definite local signs of the fistula, such as thrill or murmur. In all, the fistula was demonstrated and successfully removed at operation. In none was there evidence of recurrence, nor was the postoperative course seriously complicated.

The results of the cardiac output determinations and related observations are recorded in Table I. We have considered the value, or average of several values, obtained 10 days or more after removal of the fistula, as the normal resting output for each patient. Using this normal value for comparison, the preoperative cardiac outputs are seen to range from 127 per cent above to 21 per cent below the normal resting cardiac output (Figure 1). Our experience has been that in normal subjects a variation of as much as 25 per cent above or below a value selected as the resting cardiac output may be expected on repeated measurements on different days.

As can be seen from Table I, the alteration in cardiac output is predominantly the result of change in stroke volume rather than pulse rate.

TABLE I

*Observations on 47 patients with arteriovenous fistulas before and after operation*

| Location of fistula and number of patients in group | Duration of fistula | Time of observation | Pulse rate | Stroke volume | Cardiac index | Change in heart size | Change in blood volume |
|---|---|---|---|---|---|---|---|
| | *months* | *days before and after operation* | *beats per min.* | *cc.* | *liters per min. per sq. m.* | *cm.* | *cc. per sq. m.* |
| Carotid | 4.9 | 6 before | 73 | 116 | 4.5 | −0.5 | −440 |
| *(5)* | | 14 after | 70 | 80 | 3.7 | | |
| Vertebral | 8 | 1 before | 81 | 114 | 4.5 | −0.2 | |
| *(1)* | | 18 after | 78 | 93 | 3.7 | | |
| Subclavian | 3.3 | 19 before | 71 | 114 | 4.0 | +1.2 | −155 |
| *(3)* | | 4 after | 71 | 80 | 2.8 | | |
| Axillary | 11.3 | 13 before | 65 | 145 | 4.9 | | −200 |
| *(3)* | | 14 after | 78 | 104 | 4.5 | | |
| Brachial | 5 | 5 before | 74 | 122 | 4.3 | −0.2 | + 40 |
| *(4)* | | 14 after | 66 | 94 | 3.9 | | |
| Iliac | 7.2 | 15 before | 84 | 115 | 7.1 | −0.6 | −453 |
| *(3)* | | 20 after | 72 | 80 | 3.2 | | |
| Femoral | 5 | 8 before | 82 | 115 | 6.0 | −1.2 | −304 |
| *(12)* | | 16 after | 73 | 94 | 3.9 | | |
| Popliteal | 9.6 | 9 before | 66 | 134 | 4.8 | −0.6 | −210 |
| *(5)* | | 21 after | 65 | 103 | 3.6 | | |
| Tibial | 8.2 | 6 before | 67 | 107 | 4.0 | −0.2 | −123 |
| *(11)* | | 23 after | 70 | 94 | 3.5 | | |

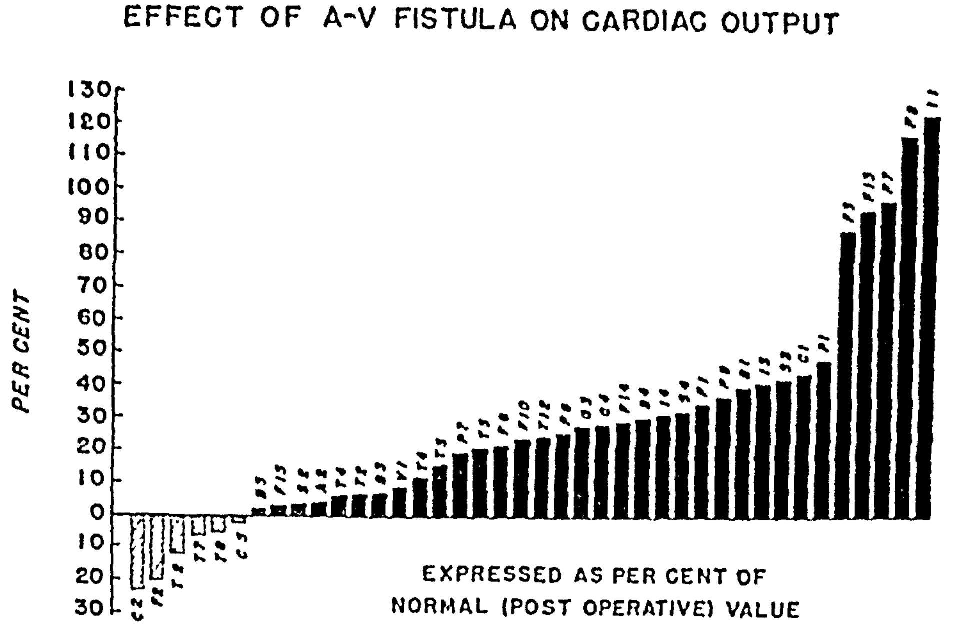

Fig. 1. The Effect of Removal of an Arteriovenous Fistula on the Cardiac Output

In only seven patients with the fistula intact was the basal pulse rate above 85 beats per minute. In four of these the fistula involved the femoral vessel; in two, the iliac; and in one, the subclavian. The cardiac output in this group of patients averaged 82 per cent above the postoperative level and ranged from 32 per cent to 127 per cent.

The arterial blood pressure showed little variation from the preoperative to postoperative period. In general, if change occurred there was a tendency toward diminution of the pulse pressure postoperatively, due predominantly to an elevation of the diastolic pressure.

In those instances where satisfactory measurements were obtained, the changes in transverse diameter of the heart and blood volume are recorded. These are discussed in detail elsewhere (21).

## DISCUSSION

Our patients were apparently healthy young adults before injury. Although the person to person variability in cardiac output is large, our study could have been made using an average normal value for comparison with the value found in the presence of the fistula. We were fortunate, however, in that our patients returned to a normal state after operation without evidence of residual abnormality in the circulation. The postoperative cardiac index varied considerably from patient to patient, but the range was similar to that found in a comparable group of normal subjects studied by the catheter method (22). Therefore, we have utilized the value obtained postoperatively for each individual as his normal and have used this value as the basis of estimating the load placed on the heart by the fistula.

The resting cardiac output with the fistula intact varied from 21 per cent below the postoperative value to 127 per cent above. A variation of 25 per cent above and below the postoperative value might be expected in any group of patients so studied and, therefore, could not be attributed to the effect of the fistula. In 22, or 47 per cent, of the patients, the change in cardiac output fell within this ± 25 per cent range. In these patients the fistula was not large enough to produce a significant change in cardiac output. In all of the other patients (25, or 53 per cent), the cardiac output with the fistula open was more than 25 per cent greater than the postoperative cardiac output, the values ranging from 25 per cent to 127 per cent above the normal output. We believe that these increases of more than 25 per cent in cardiac output are significant. This indicates that about one-half of our patients with arteriovenous fistula had a significantly elevated cardiac output as measured under basal conditions.

The increased cardiac output was primarily the result of an increase in stroke volume. The postoperative basal pulse rate was above 85 beats per minute in only seven patients. In these patients, all with clinical evidences of a large fistula, the cardiac output was markedly elevated, averaging 82 per cent above the postoperative level.

## SUMMARY AND CONCLUSIONS

A study of the cardiac output in 47 patients with arteriovenous fistulas was made by means of the low frequency critically damped ballistocardiograph before and after operative removal of the fistula. In 22 (47 per cent) of the patients there was a change of less than 25 per cent in the cardiac output in the presence of a fistula, when compared with the later normal values obtained for each patient after operative removal of the fistula. This change was not considered a significant one.

In 25 (53 per cent) of the patients with the fistula intact, there was elevation of the cardiac output of from 25 to 127 per cent above the postoperative (normal) level. Although, in general, these patients were the ones with clinical evidences of large fistulas, there was no striking correlation with the change in cardiac size, blood volume, or pulse rate, nor with duration of the fistula.

The evidence at hand indicates that the increased cardiac output is not due to an increase in filling pressure presented to the right side of the heart, but rather is related to change in the arterial tree.

Misses Maurine Giese and Margaret Steele gave valuable technical assistance.

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# -THE EFFECT OF TEMPORARY OCCLUSION OF ARTERIOVENOUS FISTULAS ON HEART RATE, STROKE VOLUME, AND CARDIAC OUTPUT[1]

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(Submitted for publication October 21, 1950; accepted, November 20, 1950)

In patients with an arteriovenous fistula temporary occlusion of the fistula results in several alterations in the circulation. The most commonly recognized of these is the slowing of the heart rate. This response, described originally in 1875 by Nicoladoni (1), was reported independently in 1890 by Branham (2). It is now almost universally referred to as Branham's sign. Subsequently, additional changes in arterial pressure (3), cardiac output (4, 5) and peripheral blood flow (6) have been noted by other authors. In the course of our studies on the cardiovascular dynamics in patients with arteriovenous fistulas we have made further observations on Branham's sign and related phenomena. These data are the subject of the present report.

## METHODS

The critically damped ballistocardiograph described by Nickerson and Curtis (7) was used in the determination of heart rate, stroke volume, and cardiac output. The method of its use is described in a separate report (5). Only patients with arteriovenous fistulas of the extremities were studied. The fistula was obliterated temporarily by rapid inflation of a blood pressure cuff to 200 mm. mercury pressure from a large air reservoir. The cuff was placed around the extremity in such a way that deflation did not affect the venous blood flow. This was done several times before taking the ballistic records in order to familiarize the patient with the procedure and to ascertain if there was discomfort upon application of pressure in the cuff. Ballistic tracings and arterial blood pressure measurement (determined by the auscultatory method) were obtained with the fistula both open and occluded. At least two sets of observations were made on each patient. In some instances 2 mgm. of atropine sulfate were injected intravenously, and the observations were repeated at the height of the atropine effect. Additional studies were made following operative removal of the arteriovenous fistula.

## RESULTS

Twenty-five patients with arteriovenous fistulas resulting from trauma were studied. The studies were restricted to those patients with fistulas of the extremities which could be occluded by inflation of a blood pressure cuff. None of the patients had clinical evidence of cardiac failure, or any disease which might alter the responses of the cardiovascular system. All of the patients reported here have been included in a previously reported study on the cardiac output in patients with arteriovenous fistulas (5).

Observations on the effect of sudden occlusion of an arteriovenous fistula are recorded in Table I and shown graphically in Figure 1. In 17 pa-

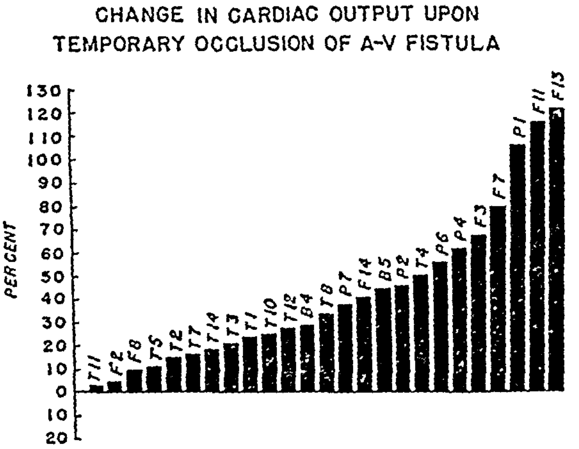

FIG. 1. CHANGES IN CARDIAC OUTPUT UPON TEMPORARY OCCLUSION OF A–V FISTULA

The cardiac output with the fistula open is expressed in per cent deviation from the cardiac output with the fistula occluded.

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[1] The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the Emory University School of Medicine, Atlanta, Georgia.

TABLE I

*Summary of observations on 25 patients with arteriovenous fistulas of the extremities*

| Location of fistula and number of patients in group | Duration of fistula | Time of observation | Comment | Cardiac index | Stroke volume | Pulse rate | Arterial pressure |
|---|---|---|---|---|---|---|---|
| | *months* | *days before and after operation* | | *liters per min. per sq. m.* | *cc.* | *beats per min.* | *mm. Hg* |
| Brachial (*2*) | 4 | 4 before | Cuff off | 4.8 | 126.5 | 70.4 | 128/79 |
| | | 5 before | Cuff on | 3.4 | 115.5 | 54 | 125/80 |
| | | 9 after | | 4.0 | 105.9 | 68.8 | 122/66 |
| Femoral (*7*) | 4.1 | 6 before | Cuff off | 5.7 | 132.8 | 77.4 | 119/90 |
| | | 4 before | Cuff on | 3.6 | 101.9 | 61.4 | 118/73 |
| | | 15 after | | 3.5 | 93.0 | 69.8 | 115/74 |
| Popliteal (*5*) | 9.6 | 9 before | Cuff off | 4.9 | 135.4 | 65.9 | 118/57 |
| | | 11 before | Cuff on | 3.7 | 102.5 | 55.8 | 117/81 |
| | | 17 after | | 4.8 | 103.8 | 65.6 | 115/48 |
| Tibial (*11*) | 8.2 | 6 before | Cuff off | 4.0 | 107.5 | 67.8 | 122/75 |
| | | 7 before | Cuff on | 3.2 | 91.9 | 63.4 | 124/79 |
| | | 23 after | | 3.6 | 95.5 | 68.4 | 114/66 |

tients (68 per cent) the heart rate decreased more than 4 beats per minute and in one case as much as 32 beats per minute. In the remaining eight cases the rate changed only 4 or less beats per minute. In 19 patients (76 per cent) stroke volume decreased 10 cc. or more per stroke. The effects on the cardiac index (cardiac output in liters per minute per square meter of body surface) varied from no change to a decrease of 3.6 liters. In 22 patients (88 per cent) it decreased 0.5 liters

TABLE II

*Observation of the effect of temporary occlusion of an arteriovenous fistula before and after administration of atropine*

| Location of fistula patient | Comment | Cardiac output | Cardiac index | Stroke volume | Heart rate | Arterial pressure |
|---|---|---|---|---|---|---|
| | | *liters per min.* | *liters per min. per sq. m.* | *cc.* | *beats per min.* | *mm. Hg* |
| Femoral (*7*) | Cuff off | 10.3 | 5.8 | 124 | 83 | 110/60 |
| | Cuff on | 6.5 | 3.7 | 107 | 61 | 115/70 |
| | Atropine—2 mgm. intravenously | | | | | |
| | Cuff off | 12.3 | 7.0 | 123 | 100 | 125/85 |
| | Cuff on | 8.6 | 4.9 | 83 | 104 | 145/95 |
| Femoral (*13*) | Cuff off | 12.0 | 6.5 | 158 | 76 | 120/55 |
| | Cuff on | 6.1 | 3.3 | 139 | 44 | 125/70 |
| | Atropine—2 mgm. intravenously | | | | | |
| | Cuff off | 9.7 | 5.3 | 105 | 93 | 130/85 |
| | Cuff on | 7.9 | 4.3 | 84 | 94 | 150/90 |
| Popliteal (*4*) | Cuff off | 11.6 | 6.0 | 140 | 83 | 115/60 |
| | Cuff on | 6.8 | 3.5 | 110 | 62 | 115/70 |
| | Atropine—2 mgm. intravenously | | | | | |
| | Cuff off | 15.6 | 8.0 | 127 | 123 | 135/90 |
| | Cuff on | 12.1 | 6.2 | 102 | 119 | 140/100 |
| Popliteal (*6*) | Cuff off | 5.8 | 3.6 | 93 | 62 | 110/60 |
| | Cuff on | 4.2 | 2.6 | 70 | 59 | 115/75 |
| | Atropine—2 mgm. intravenously | | | | | |
| | Cuff off | 8.8 | 5.5 | 86 | 102 | 120/65 |
| | Cuff on | 6.4 | 4.0 | 62 | 102 | 130/85 |
| Tibial (*4*) | Cuff off | 9.4 | 5.0 | 132 | 71 | 120/60 |
| | Cuff on | 6.9 | 3.6 | 120 | 57 | 120/80 |
| | Atropine—2 mgm. intravenously | | | | | |
| | Cuff off | 9.8 | 5.2 | 100 | 98 | 140/90 |
| | Cuff on | 5.6 | 3.0 | 59 | 96 | 160/100 |

or more, which, under the conditions of the study, probably represents a minimal detectable change. Changes in arterial pressure were not large, but almost uniformly consisted of an increase in diastolic pressure and frequently an increased systolic pressure upon occlusion of the fistula.

Additional studies on the effect of compression of the fistula were made on five patients who had received intravenously 2 mgm. of atropine sulfate (Table II). In all the atropine produced an increased pulse rate and in some an increase in cardiac index above the basal level. Upon inflation of the cuff the change in pulse rate was not greater than 4 beats per minute. Despite this relatively small change in heart rate, the decrease in stroke volume varied from 21 to 41 cc. and in cardiac output from 1.8 to 4.2 liters.

## DISCUSSION

The advantages of the ballistic method for determination of relatively rapid changes in cardiovascular dynamics have been enumerated previously (5, 8). One disadvantage in the use of the ballistocardiograph is that manual compression of the fistula interferes with the movements of the ballistic bed. Therefore, a blood pressure cuff was used. This means of temporarily occluding the fistula occludes not only the fistula but also all of the circulation to the extremity distal to the cuff.

Studies on normal subjects, and on normal extremities of patients with arteriovenous fistula elsewhere in the body, have shown that rapid inflation of the cuff to 200 mm. mercury pressure produced practically no change in cardiac output or pulse rate. In one patient, in whom the fistula was also occluded with a Matas compressor (compression of only the major vessel entering the fistula) (9), it was found that the cuff and compressor produced identical changes in heart rate and cardiac output.

The findings reported here confirm Branham's observations. The rapidity of the reaction suggests that a nervous reflex is involved. Lewis and Drury (6) observed that they could block the reaction by the preliminary administration of atropine. Rieder (10) was unable to confirm this, but it is likely that his dosage of atropine was inadequate. The present results confirm the findings of Ellis and Weiss (11), and of Kramer and Kahn (12), that atropinization can block the change in pulse rate. This effect of atropine was interpreted as evidence that the pulse slowing response is mediated by a nervous mechanism, and by the vagus nerve in particular.

The specific stimulus which activates the bradycardiac response is not completely understood. It has been suggested that the vascular bed in and around the fistula may take on a function similar to the carotid sinus or aortic depressor areas in that local pressure changes are reflected in reflex changes in cardiac function. This does not appear to be the case. Observations (13, 14) have revealed that the pulse rate changes occur in patients with high spinal anesthesia. In these patients the area of the fistula is probably devoid of functional nervous connections, but it is not certain that complete denervation is present.

Several other systemic circulatory alterations take place when an arteriovenous fistula is occluded. Measurements of the pressure in the right atrium have failed to show any alteration, whereas the arterial pressure is found to rise. The latter phenomenon offers the most likely explanation for the slowing of the pulse rate. It is an established physiologic principle (Marey's law) that the pulse slows when the blood pressure rises. The origin of this type of reflex response appears to occur in the pressure sensitive end organs in the carotid sinus and aortic arch. In addition, experience in normal human subjects has shown that drugs which cause an increased arterial pressure with little change in cardiac output, such as paredrine, usually cause a slowing in the heart rate (15).

The present observations also demonstrate a striking decrease in stroke volume upon compression of the fistula. As a result of the summation of changes in heart rate and stroke volume the cardiac output is reduced. However, the reduction in stroke volume appears to be independent of the pulse rate change. Atropine, which abolishes the heart rate change, does not prevent the reduction in stroke volume. In fact, in the cases observed, the stroke volume reduction in occlusion of the fistula is greater with atropine than without. This effect is probably the result of the shorter filling time which is a consequence of the higher heart rate during atropinization.

The nature of the mechanism responsible for the change in stroke volume upon opening or closing the fistula is of considerable interest. The change occurs rapidly, significant alterations appearing within the first few heart beats. Theoretically it appears that the stroke volume may be altered by one of three mechanisms: changes in the pressure gradient filling the ventricles, variation in the completeness of systolic emptying of the ventricles, and variation in the degree of diastolic relaxation of the ventricles.

Studies using the method of right heart catheterization have revealed evidence regarding the first possibility. It has been demonstrated (16) that there is no change in the mean right atrial pressure when the fistula is opened and closed, although there may be large changes in stroke volume. Changes in atrial pressures do not then appear to be the cause of the increased stroke volume. Nevertheless, one qualification remains in that a pressure gradient cannot be determined by measuring the pressure at a single point in the system. Pressure in the ventricle may change, hence the gradient filling the ventricle would be altered.

There is, however, no direct evidence regarding the completeness of systolic emptying and the changes in diastolic relaxation of the ventricles in normal man. In addition to invoking the passive increase of fluid movement on opening the fistula as an explanation of the increased stroke volume there is the possibility of involvement of some nervous mechanism. Such a mechanism may influence the degree of relaxation of the ventricles, and the necessary stimulus could originate in the venous bed. If a nervous reaction is involved, it must occur rapidly and not be blocked by atropine.

Changes in cardiac output and stroke volume similar to these reported here may be observed in normal subjects by the production of large areas of reactive hyperemia (16). If blood pressure cuffs are placed around the upper thighs and inflated to 200 mm. mercury pressure, little change in heart rate and cardiac output is produced. If the tourniquets are kept on for 20 minutes, and observations are made at the time of release, striking changes in heart rate and output may be noted. The heart rate increases, and the cardiac output per minute almost doubles. These changes, as those in our patients with arteriovenous fistulas, occurred without significant alterations in atrial pressure.

After operative removal of the fistula the cardiac output was found to be nearly the same as that observed previously upon temporary compression of the fistula. Cardiac output is such a variable function, changing markedly with even emotional factors, that it is difficult to say whether the failure of the values to coincide exactly always represents a real difference in output as related to the length of time that the fistula is closed, or whether it represents variations in the conditions under which the values were obtained.

## SUMMARY AND CONCLUSIONS

The changes in heart rate and output occurring upon temporary compression of an arteriovenous fistula have been studied in a group of 25 patients.

In 68 per cent of the patients there was a significant decrease in heart rate (Branham's sign) which occurred almost immediately upon compression of the fistula and which was blocked by use of atropine.

In 88 per cent of the patients there was a decrease in stroke volume and cardiac output which occurred rapidly and simultaneously, but which was not altered by the use of atropine.

The evidence indicates that the change in heart rate results from a nervous reflex mediated by the vagus nerve, and most likely arises from alterations in arterial pressure. It appears that the change in stroke volume is due to variations in diastolic relaxation of the ventricle or to variation in the completeness of systolic emptying.

Although exactly comparable observations are difficult to obtain, the cardiac output found immediately upon closure of an arteriovenous fistula is approximately the same as that found several weeks after operative removal of the fistula.

Misses Mary Margaret Steele and Maurine Giese gave valuable technical assistance.

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# THE BLOOD VOLUME IN PATIENTS WITH ARTERIOVENOUS FISTULAS[1]

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(Submitted for publication October 21, 1950; accepted, November 20, 1950)

There has been much controversy concerning the changes in blood volume which occur in the presence of arteriovenous fistulas. The large number of vascular injuries treated at Ashford General Hospital during the recent war offered an unusual opportunity to study this problem. This paper reports observations on the blood volume changes in a group of 41 such patients studied before and after operative removal of an arteriovenous fistula.

In 1924 Holman (1) reported his studies on animals with arteriovenous fistulas. He used the brilliant vital red method of blood volume determination, obtaining a single dyed specimen four minutes after the injection of the dye. He found that the blood volume increased in the presence of a fistula. Later he reported similar blood volume studies in patients with arteriovenous fistulas (2, 3); in most instances there was a reduction in blood volume following removal of the fistula. In their studies on blood volume Rowntree and Brown (4) included seven patients with arteriovenous fistulas, three of whom had congenital lesions. Although the average blood volume was slightly above the normal value, in only one patient was there a distinctly increased blood volume. Pemberton and Saint (5) reported finding a slightly elevated value in a single patient with a congenital communication between artery and vein. Reid and McGuire (6) expressed doubt regarding the blood volume changes in view of their findings on three patients studied before and after operation. In one patient there was essentially no change in blood volume; in another, a postoperative decline of 600 cc.; and in the other, a postoperative drop of 1,000 cc. and nine months later, a return to the preoperative volume. In two experimental animals they found that a slight increase in blood volume accompanied the fistula. Using the blue dye T-1824 in a single case, Ellis and Weiss (7) found the blood volume within normal limits, but they did not repeat the measurement postoperatively. Kennedy and Burwell (8) reported a large value in a patient with multiple communications between artery and vein. The volume decreased after operation, but later fluctuated considerably as the fistula recurred.

There are many theoretical and practical objections to the methods used in the earlier studies and more recent observations have been inadequate in number. Therefore, the studies reported here were made.

## METHODS

All determinations reported here were made on patients under basal conditions, at least 12 hours after the last meal and before arising in the morning. The plasma volume was measured using the blue dye T-1824 recommended by Gregersen, Gibson, and Stead (9). All blood samples were taken from the antecubital veins, using a dry syringe, and avoiding the hemoconcentrating effect of a tourniquet. After obtaining a dye-free sample, 3 cc. of an 0.1 per cent solution of the dye was injected from a calibrated syringe, and the syringe was rinsed three times with blood. Care was taken to prevent and note any extravascular loss of the dye. Exactly 10 minutes after the dye injection, a dyed sample of blood was obtained, again with precautions against hemolysis and hemoconcentration. In some patients, but not all, an inlying needle was left in place and serial samples obtained in order to determine the rate of disappearance of the blue dye.

The blood samples were placed in small tubes under a layer of mineral oil, allowed to clot and retract, then were centrifuged gently. The samples were discarded if there was evidence of lipemia or hemolysis. A Coleman Junior Spectrophotometer or a Nickerson decade photometer (10) was used in determining the concentration of

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[1] The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the Emory University School of Medicine, Atlanta, Georgia.

the dye T-1824. This was done by measuring the light absorption of the dyed serum samples relative to the dye-free sample at a wave length of 620 millimicrons. Previously the apparatus had been calibrated with known amounts of dye in serum.

Blood specimens were also obtained for hematocrit reading, hemoglobin, and total protein determinations. Heparin was used as the anticoagulant in the hematocrit tubes. The hemoglobin concentration was determined by measuring the optical density of a dilute alkaline solution of blood. Total protein was determined by the density method of Barbour and Hamilton (11).

The plasma volume was calculated from either the dye concentration value obtained from extrapolation of the disappearance curve plotted semilogarithmically (12), or from the single 10-minute point as suggested by Gregersen (13). The total blood volume was determined from the plasma volume and the hematocrit reading. To facilitate comparison between patients of different size, the blood volume per square meter of body surface was calculated.

Additional studies, reported in detail elsewhere (14, 15), were made on many of these patients. Included were roentgenographic estimations of the heart size (obtained by measuring the transverse diameter of the heart on a teleoroentgenogram), and cardiac output determinations (by the ballistic method).

## RESULTS

Observations were made on 41 patients, and the essential findings are reported in Table I. All patients had arteriovenous fistulas resulting from trauma. As far as could be determined, all the patients were healthy young adults before injury, and none had complicating injuries or illness that might have altered the blood volume. There was one woman in the group. Table I shows that the location of the lesions varied considerably. In all patients there were characteristic physical signs of the fistula. The duration between removal of the fistula and the infliction of the wound which caused it varied from slightly more than two months to two years. In no patient was there evidence of frank cardiac failure. The operative removal of the fistula was done with either sodium pentothal or continuous spinal anesthesia. In all instances the fistula was successfully removed, and the postoperative course was uneventful. With the exception of those determinations done on the third postoperative day, the patients were usually allowed out of bed by the time the blood volume determinations were made.

In the entire group, before operation the blood volume per square meter of body surface ranged from 2,400 cc. to 4,030 cc. Ten days or more after operation it ranged from 2,330 cc. to 3,380 cc. In 23 patients (56 per cent) the change was less than 200 cc. A change of 200 cc. or less was not considered a significant change.

TABLE I

*Summary of observations on 41 patients with arteriovenous fistulas*

| Location of fistula and number of patients in group | Duration of fistula | Time of observation | Plasma volume | Total blood volume | Deviation from normal blood volume | Hematocrit reading | Total plasma protein | Heart size |
|---|---|---|---|---|---|---|---|---|
| | *months* | *days before and after operation* | *cc.* | *cc.* | *cc. per sq. m.* | | *gms. per 100 cc.* | *cm.* |
| Carotid | 3.5 | 1 before | 2650 | 5100 | | 48.2 | 6.7 | −1.5 |
| (*1*) | | 21 after | 2420 | 4385 | −440 | 44.8 | 6.1 | |
| Subclavian | 3.5 | 7 before | 2848 | 5540 | −168 | 49.1 | 6.0 | +0.5 |
| (*4*) | | 12 after | 2932 | 5387 | | 46.1 | 6.4 | |
| Axillary | 8.1 | 12 before | 2990 | 5700 | −122 | 47.7 | 6.3 | −5.6 |
| (*5*) | | 20 after | 2806 | 4947 | | 46.8 | 6.4 | |
| Brachial | 3.5 | 6 before | 2620 | 5053 | 90 | 47.8 | 6.4 | 0 |
| (*3*) | | 10 after | 2970 | 5220 | | 49.4 | 6.5 | |
| Iliac | 6 | 14 before | 3146 | 5616 | −365 | 44.3 | 6.9 | −0.6 |
| (*4*) | | 14 after | 2667 | 4957 | | 46.4 | 6.3 | |
| Femoral | 6.1 | 6 before | 2972 | 5645 | −238 | 47.6 | 6.2 | −1.2 |
| (*13*) | | 14 after | 2618 | 5169 | | 49.4 | 6.4 | |
| Popliteal | 6.8 | 7 before | 2827 | 5584 | − 76 | 49.2 | 6.4 | −0.2 |
| (*5*) | | 15 after | 2856 | 5476 | | 47.7 | 6.8 | |
| Tibial | 8.7 | 3 before | 2735 | 5068 | −116 | 45.6 | 6.2 | −0.4 |
| (*6*) | | 19 after | 2661 | 4865 | | 45.0 | 6.5 | |

In 18 patients (44 per cent), after removal of the fistula there was a decrease in blood volume from 200 cc. to 1,060 cc. per square meter body surface. In many instances these changes were confirmed by repeated observations. On the other hand, in none of the patients was there a significant increase in blood volume following operation.

The hematocrit readings varied slightly, most often showing a tendency to fall somewhat after operation. Therefore, the calculated red blood cell volume underwent similar changes. The hemoglobin and total protein concentration did not undergo marked alteration. The cardiac output data and changes in heart size have been described in detail elsewhere (14).

There has been wide use of the blue dye T-1824 (9) in the determination of plasma volume. In the present studies the disappearance of the dye was plotted semilogarithmically as recommended by Gregersen and Rawson (12). More recently Gregersen (13) has suggested that in certain situations a single dyed specimen obtained 10 minutes after dye injection is almost as reliable as an extrapolated value for dye concentration obtained from the disappearance curve of the dye. He found this true in normal patients and patients in shock. In the case of many patients reported here there were obtained both 10-minute samples and multiple specimens for plotting the disappearance slope of the dye. There was no evidence that the disappearance rate of the dye was affected by the presence of the arteriovenous fistula, and the plasma volume as calculated by the two methods did not differ significantly. It must be remembered, however, that none of these patients had frank congestive heart failure. It is possible that the prolonged mixing time of cardiac failure might invalidate the 10-minute point method. Because of the limited time that the patients were available, and because multiple determinations were made on a large group, the 10-minute point method was used extensively.

The calculation of the total blood volume from the hematocrit reading and the plasma has been subject to criticism (16, 17). This is primarily because the peripheral venous hematocrit reading is imperfect from the standpoint of indicating the proportion of cells in the blood in flow, and because the plasma trapped among the cells in centrifugation prevents arrival at a true cell-plasma ratio by hematocrit reading. Despite the handicaps, a value determined by this means may serve many useful purposes. It appears particularly useful in measurements, such as these reported here, where comparative observations are made on the same person who has minimal hematocrit changes.

Because it appears to be at the expense of the ratio of cells to plasma that the body maintains the total blood volume at a constant level, we found the total blood volume the most stable and useful value for these studies. Since the body can mobilize fluid and protein with relative rapidity, the plasma volume appears to vary to compensate for changes in the red cell volume. Studies on the effect of simple hemorrhage show that these changes in plasma volume first occur with a slow readjustment of the red cell volume and hematocrit reading to the normal value (18). Similar changes may be seen soon after an operation, with its accompanying blood loss and dehydration. Because of this, the plasma volume is subject to much more fluctuation than is the total blood volume, and knowledge concerning it was of less value in these studies than was evidence of changes in the total blood volume. Although some of the postoperative measurements reported here were made relatively early following operation, an effort was made to delay them long enough to avoid any immediate effects of the operation. Only observations made 10 days or more after operation were used in the calculation of the normal values. Almost all of the patients were ambulatory by this time. The values obtained fell in the same range as those reported previously for normal male subjects, the average blood volume being near 2,800 cc. per square meter of body surface (19).

The results of these studies are expressed graphically in Figure 1, considering the postoperative blood volume as the normal for each patient. A majority of the patients showed a change of less than 200 cc. in blood volume per square meter body surface. This degree of change (about 7 per cent of the normal value) may be expected independently, without direct effect on the arteriovenous fistula. Such factors as technical errors in blood volume determination, changes resulting from operation, and bed rest could readily account for a 7 per cent variation. On the other hand, following operation, although no patient had an in-

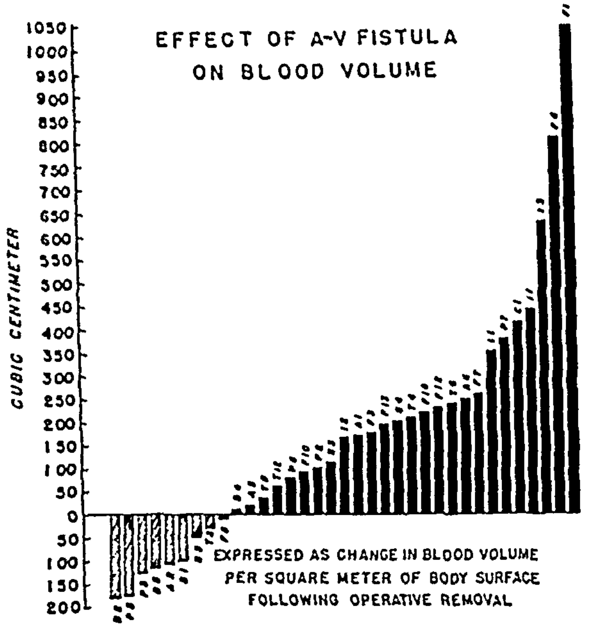

Fig. 1. Effects of an A–V Fistula on Blood Volume

The change in blood volume from before operation to the postoperative period is expressed in cubic centimeters per square meter of body surface.

crease in blood volume greater than 200 cc., 18 patients (44 per cent) had a decrease of more than 200 cc. Insofar as could be determined, this decrease was not related to any technical errors or to any incident of the operation or convalescence not directly related to the removal of the arteriovenous fistula. In several instances repeated observations before and after operation verified the presence of the change, and revealed that the decline was in progress by three days postoperatively, and was apparently complete by 10 days. Observations were not made earlier than three days postoperatively because it was felt that changes incident to the operation might cause alterations in blood volume not due to the actual removal of the fistula.

The findings reported here are in agreement with earlier reports that blood volume may be significantly elevated in patients with arteriovenous fistulas. It is not a consistent reaction, however, as changes were noted in only less than half of the patients studied. In an attempt to elucidate the cause of this blood volume change, an effort was made to relate the blood volume change with other alterations in the circulation.

It appears that the size of the fistula is a major factor in determining the increase in blood volume. For instance, two of the four patients with the greatest change in blood volume had fistulas involving the iliac vessels. However, the actual size of a fistula is difficult to determine. Clinical evidences, such as the size of the vessels involved and the intensity of the thrill or murmur, give some evidence, although not entirely accurate, regarding the magnitude of the shunt. Actual measurement of the fistulous opening upon operation has not proved successful because of the variable de-

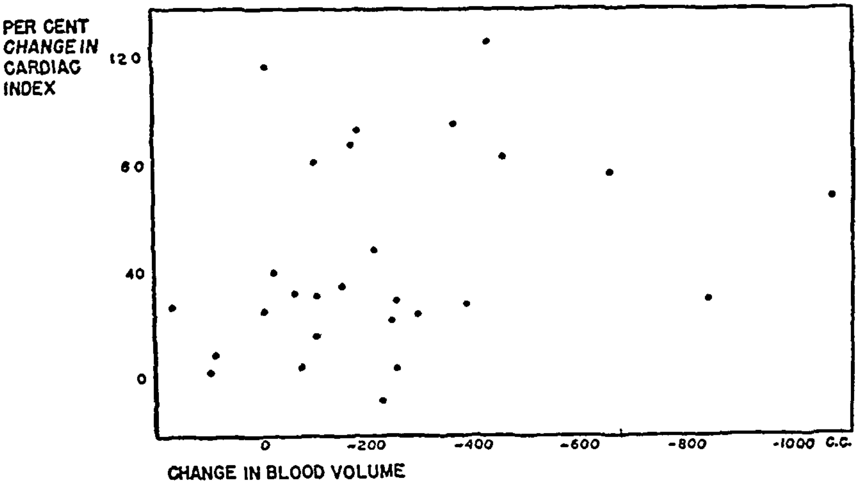

Fig. 2. A Chart Demonstrating the Relationship Between the Change in Cardiac Output and the Change in Blood Volume Following Removal of an A–V Fistula

The change in blood volume is expressed in cubic centimeters per square meter of body surface.

gree of vascular spasm. Measurement of the opening after removal from the body is difficult for the same reason plus the shrinking effect of tissue fixation.

In many patients with arteriovenous fistulas the resting level of cardiac output is above normal (14). Since the elevation of the cardiac output above normal might be proportional to the size of the fistula, correlation of the increase in blood volume with the increase in cardiac output was attempted (Figure 2). To avoid the considerable range in basal cardiac output from person to person, the blood volume change was plotted against the per cent of deviation from normal of the cardiac output in the particular patient observed. In other words, if the preoperative cardiac output was twice that found as the postoperative basal value, a 100 per cent increase in cardiac output was plotted. The correlation coefficient was 0.26, a value which indicated that the correlation was not good. It appears, however, that the size of the fistula may be at least one of several factors in the changes leading to an increased blood volume.

It has repeatedly been observed that in patients with arteriovenous fistulas the heart size, determined roentgenologically, may become abnormally large, and after the fistula is removed, it returns to normal size (20). Since there is evidence to suggest that the heart size is related to the blood volume, these two findings were correlated. Twenty-nine of the patients had satisfactory teleoroentgenograms of the heart made before and after operation. In many instances there were repeated observations. The change of blood volume was plotted against the change in the transverse diameter of the heart in Figure 3. The correlation coefficient of value 0.36 indicates that a significant degree of correlation was not present. This is not entirely surprising in view of recent evidence that in normal subjects the rapid increase in blood volume of about 1,000 cc. failed to produce a distinct change in heart size (21). Holman found a change in heart size in dogs upon increasing or decreasing the blood volume, but he studied changes of much greater magnitude than seen in patients with arteriovenous fistulas (1). A change of 500 cc. in a dog such as he studied is equivalent to a change of approximately 4,000 cc. in a human. In neither of the afore-mentioned studies was the increase in blood volume accompanied by an increase in extracellular fluid volume, which if present might alter the situation considerably. It appears likely that other factors, such as the increased demand on the heart, play a major role.

An attempt was made to relate the increase in blood volume to the duration of the fistula. Our data are not satisfactory for this purpose, however, because so few patients had large fistulas of long duration. Most of the patients with lesions of con-

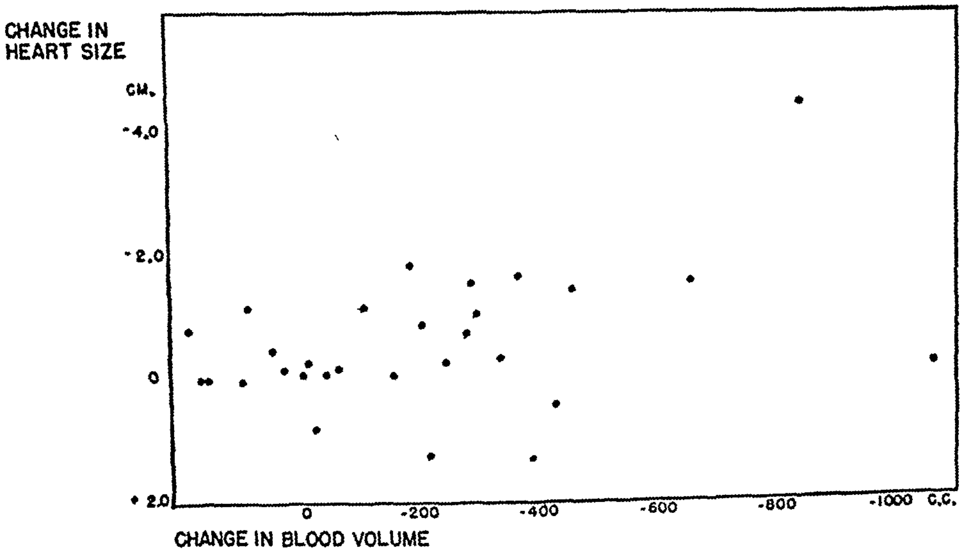

FIG. 3. THE CHANGE IN BLOOD VOLUME PLOTTED AGAINST THE CHANGE IN HEART SIZE

The change in heart size is expressed as the change in transverse diameter of the heart determined from the teleoroentgenogram.

siderable age had rather small fistulas which had often escaped notice by the patient and physician.

Our results demonstrate that in some patients with arteriovenous fistulas there is a significant increase in blood volume. After operative removal of the fistula, the blood volume, if elevated, returns to normal. Since the basic factors controlling blood volume in the normal person are so little understood, it is not surprising that we cannot explain the increased blood volume in these patients.

It is possible that with some large venous channels engorged with blood, the mechanisms controlling the blood volume might be misled. Since the amount of blood remaining in the rest of the vascular bed would be less than normal, increased production of blood might be stimulated. This could be the basic mechanism causing the increased blood volume. There is also the possibility that the increased venous pressure in the region of the fistula may dilate the vascular bed in that region, thus increasing its capacity for blood and spurring the demand for more blood.

It has been suggested that the increase in blood volume seen in patients with arteriovenous fistulas merely represents the increase in blood volume seen in patients with any type of congestive heart failure (6); that is, it may be the increase in blood volume of cardiac failure rather than that of arteriovenous fistula *per se*. Although none of the patients observed in these studies developed the complete clinical picture of cardiac failure, it cannot be denied that there may be a similar mechanism in the causation of their increased blood volume.

## SUMMARY AND CONCLUSIONS

1. Observations on the blood volume have been made before and after operative removal of an arteriovenous fistula in 41 patients.
2. In 56 per cent of the patients there were changes in blood volume of less than 200 cc. per square meter of body surface. This was not considered a significant variation.
3. In 44 per cent of the patients the decrease in blood volume after operation ranged from 200 cc. to 1,060 cc. per square meter of body surface, indicating an abnormally large blood volume in the presence of the fistula.
4. The relationship of the change in blood volume to the size and duration of the fistula, the cardiac output, and the changes in heart size have been studied. In general, the patients with an elevated blood volume were those with clinical evidences of a functionally large fistula.

Miss Maurine Giese and Miss Mary Margaret Steele gave valuable technical assistance.

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# THE EFFECT OF ADRENOCORTICOTROPHIC HORMONE ON CHILDREN WITH THE NEPHROTIC SYNDROME. II. PHYSIOLOGIC OBSERVATIONS ON DISCRETE KIDNEY FUNCTIONS AND PLASMA VOLUME[1]

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(Submitted for publication September 12, 1950; accepted, December 4, 1950)

## INTRODUCTION

Inability to predict the time of occurrence of spontaneous diureses in the nephrotic syndrome has retarded investigations of the physiologic changes which may precede, accompany, and follow these heartening but enigmatic events. It has been demonstrated recently that several agents (1–5) are capable of *inducing* diureses at a predictable time in a far higher percentage of trials than could be attributed to chance. Adrenocorticotrophic hormone (ACTH) is one such agent, and the abrupt, profuse, and sustained diureses which may accompany or follow its administration are clinically similar to the dramatic diureses which occur spontaneously. Clinical observations on the effect of ACTH[4] on a group of children with the nephrotic syndrome is the subject of a separate report (6) in which the possible clinical significance of the results is also discussed. Serial physiologic measurements were made on selected patients before, during, and after administration of ACTH. These observations were directed toward an increased understanding of the syndrome itself and especially of the mechanism of diuresis rather than toward evaluation of possible relationships between the disease and pituitary or adrenocortical function. As an integral part of the approach, an attempt was made to disclose, if possible, consistent changes which might precede the outset of diuresis and, to this end, as many relevant physiologic measurements as practicable were made. These included: blood volume; discrete kidney functions; concentrations of electrolytes and nitrogenous constituents of serum; metabolic balances of electrolytes and nitrogen; serum lipid fractions; and urinary protein and sediment.

It has not been possible from any one or combination of changes to predict when diuresis would occur in relation to administration of ACTH. The most striking changes observed after the outset of diuresis were increases in plasma volume and unexpectedly large increases in glomerular filtration rate (GFR). These changes together with observations on other discrete kidney functions are described in the present paper. Observations on other measurements will be the subject of a subsequent report (7).

## SUBJECTS AND METHODS

Observations were made on eight children with the nephrotic syndrome whose clinical status and course during ACTH administration is described in a separate report (6). Clinical data relevant to the observations presented here are included in Table I.

Various combinations of the following measurements were made before, during, and after administration of ACTH: daily weight; plasma volume and hematocrit; clearances of inulin ($C_{IN}$); endogenous creatinine ($C_{CR}$); thiosulfate ($C_{THIO}$) and p-aminohippurate ($C_{PAH}$); and maximum tubular excretion of PAH ($Tm_{PAH}$). In three children (I. C., K. N., J. S.), 24-hour clearances of endogenous creatinine ($C_{CR-24}$) were measured. These children were on low salt (20 meq. per day) but otherwise normal diets. Most of the time they were ambulatory and normally active.

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[4] We wish to thank Dr. John R. Mote of the Armour Laboratories for allocating the ACTH used in these observations.

Methods for inulin, p-aminohippurate, urea (8) and endogenous creatinine (9), in blood and urine, and the catheterization and infusion technic used for short-term simultaneous clearances have been described (8). Serum and urine thiosulfate concentrations were determined by the method of Newman, Gilman, and Phillips (10). $C_{CR-24}$ was calculated using 24-hour creatinine excretion and mid-point serum values interpolated from creatinine determinations made one to three days apart. Plasma volume was measured with T-1824 using a single 10-minute blood sample and the acetone extraction method of Chinard and Eder (11) for lipemic sera. Blood volume was calculated from plasma volume and hematocrit (12).

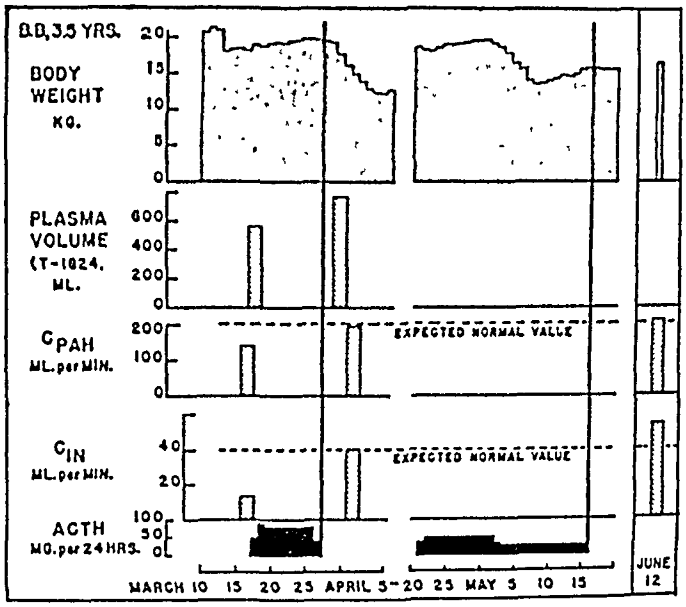

FIG. 1. RELATION OF CHANGES IN BODY WEIGHT, PLASMA VOLUME, AND CLEARANCES OF INULIN AND p-AMINOHIPPURATE TO ADMINISTRATION OF ACTH IN A CHILD WITH THE NEPHROTIC SYNDROME

## RESULTS[5]

Changes observed in discrete kidney functions and plasma volume in relation to hormone administration are given in Table I. Data from B. B. and I. C. are shown graphically in Figures 1 and 2.

### *Glomerular filtration rate*

The most striking changes observed during or following diureses associated with ACTH administration were marked increases in $C_{IN}$. Such increases were observed on eight occasions in six of the eight children and were greatest in patients with low initial values. Thus increases in $C_{IN}$ of 179, 211, 190, 50, and 256% above control values were observed respectively in I. C., K. N., B. B., M. L., and S. S.[6] In B. B., this change in $C_{IN}$ represents *an increase from 35 to 133% of normal* (Figure 1). In the three children whose initial values were within the normal range, measurements of $C_{IN}$ showed no significant increase in two (J. S. and R. T.), but revealed an increase to the high value of 123% of normal in one (R. K.). Failure to observe an increase in $C_{IN}$ following diuresis (as in I. C., February 1) may possibly be due to failure to make the measurement at the proper time as discussed later.

In an attempt to determine when changes in GFR occurred and to follow their course, 24-hour clearances of endogenous creatinine were measured. The ratio of $C_{CR} : C_{IN}$ is irregularly greater than one in children with kidney disease (17). Consequently, repeated short term measurements of this ratio were made to relate changes in the 24-hour clearance to changes in GFR. Even though changes in this ratio were taken into account, $C_{CR-24}$ was interpreted only as reflecting changes in rather than as an absolute measure of GFR. Despite these reservations, changes in $C_{CR-24}$ were sufficiently large to demonstrate that GFR increased during the first 24 hours of diuresis, as shown in Figure 2. Additional impressions concerning changes in GFR during and following ACTH administration are gained from inspection of the data from I. C.[7]

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[5] The frequency with which diuresis has been observed with ACTH in children with the nephrotic syndrome is reported separately (6). In this group of eight children, selected because measurements had been made both before and after ACTH, a diuresis failed to occur in only one of 14 courses. This failure occurred when only 50 mg. per day for three and one half days was given to a child (I. C.) who diuresed on three other occasions with larger dosages.

[6] Only a small fraction of the observed increases in $C_{IN}$ could be related to increases in rate of urine flow during diuresis.

[7] In this patient and in others receiving cortisone in larger dosages no diuresis occurred and no increase in GFR was observed. On the contrary, a marked fall in GFR was observed in S. S. 15 days after he had received 100 mg. of cortisone in seven days. There are not sufficient data to indicate the significance of this observation.

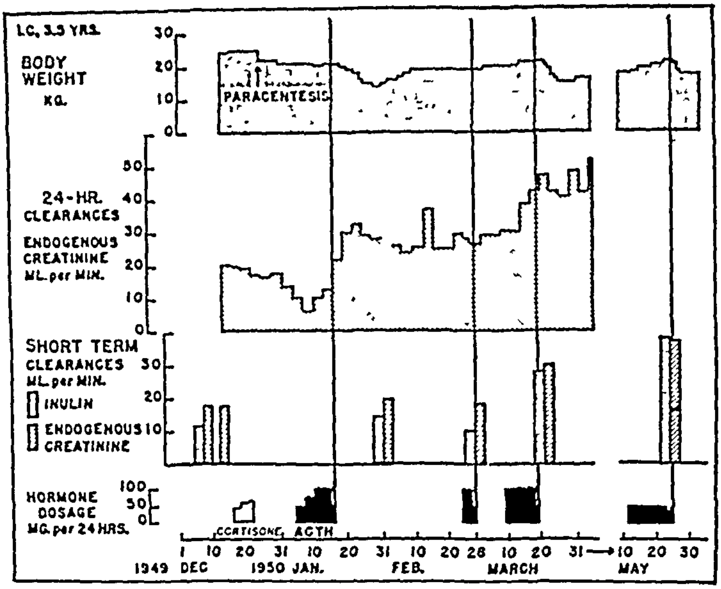

FIG. 2. RELATION OF CHANGES IN BODY WEIGHT AND CLEARANCES OF INULIN AND ENDOGENOUS CREATININE TO ADMINISTRATION OF ACTH IN A CHILD WITH THE NEPHROTIC SYNDROME

The decrease in $C_{CR-24}$ seen during the first course of ACTH administration (January 4 to 16) has been observed in other patients and was often associated with a marked rise in serum urea nitrogen and endogenous creatinine. These changes suggest a temporary fall in GFR during ACTH administration. The low value for $C_{IN}$ measured on February 28, one day after a three day course of ACTH which did not induce diuresis, lends support to this impression.[8] The pattern of changes in $C_{CR-24}$ in Figure 2 suggests that each diuresis was accompanied by an increase in GFR and followed by a decrease from the maximum value with subsequent stabilization at a value slightly or markedly above the control. It is possible that the fall in GFR may already have occurred when $C_{IN}$ was measured on February 1, 1950, since re-accumulation of edema already had begun. Repeated responses to ACTH may, however, be associated, as shown in Figures 1 and 2, with a progressive increase in GFR to normal (I. C.) or even "supernormal" values (B. B.).

Changes of this order of magnitude in $C_{IN}$ in children with diseased kidneys must raise the question of the validity of $C_{IN}$ as a measure of GFR under these conditions. This question becomes especially pertinent in view of the consistent changes observed in three children (I. C., K. N., and B. B.) in the $C_{CR} : C_{IN}$ ratio which decreased from a range of 1.5 to 1.9 before to a range of 1.0 to 1.4 after diuresis. However, the reasonably good agreement between $C_{IN}$ and $C_{THIO}$ ($C_{THIO}$: $C_{IN}$ ratios ranging from 0.9 to 1.2) at both decreased and increased values of $C_{IN}$ provides evidence that $C_{IN}$ was a measure of GFR in these children. The changes observed in the $C_{CR} : C_{IN}$ ratio are unexplained at present.

### *Effective renal plasma flow ($C_{PAH}$)*

Significant and consistent increases in $C_{PAH}$ were observed to accompany increases in $C_{IN}$ during diureses occurring with ACTH. However, increases in $C_{PAH}$ were consistently less than those in $C_{IN}$ so that in the six children (I. C., K. N., B. B., R. K., M. L., and S. S.) who showed an increase in $C_{IN}$ the $C_{IN} : C_{PAH}$ ratio increased from a range of 0.10 to 0.17 before to a range of 0.22 to 0.32 after diuresis. Whether these changes

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[8] $C_{CR}$, measured simultaneously with $C_{IN}$, showed a comparable decrease at this time. Failure of $C_{CR-24}$ to reflect this decrease may be partly explained by the inaccuracy of calculating clearances using mid-point values for serum creatinine concentrations interpolated from determinations made on fasting samples taken at intervals of several days (18). $C_{CR-24}$ was consistently higher than $C_{CR}$ which might be expected from the fact that the latter was always measured early in the day with the subject at rest and in a fasting state, whereas the former might be affected by such factors as eating and activity.

TABLE I—*Measurements of discrete kidney functions* and plasma volume in relation to hormone administration*

*The New York Hospital*

| Subject | Date | Hormone administration | Weight | $C_{IN}$ | $C_{CR}$ | $C_{THIO}$ | $C_{PAH}$ | $T_{MPAH}$ | $C_{IN}/C_{PAH}$ | $C_{CR}/C_{IN}$ | $C_{THIO}/C_{IN}$ | $C_{IN}/T_{MPAH}$ | Plasma volume | Blood volume | Hemato-crit | Comment |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | *kg.* | *ml. per min.* | *ml. per min.* | *ml. per min.* | *ml. per min.* | *mg. per min.* | | | | | *ml.* | *ml.* | *%* | |
| I. C. | 12/7/49 | | 23.4 | 11.9 | 18.0 | 12.0 | 77.9 | — | 0.15 | 1.5 | 1.0 | — | | | | Duration of disease—7 mos. |
| F. Age 3.5 yrs. | 12/12 | | 24.4 | — | 18.0 | — | — | — | — | — | — | — | | | | Constant edema since outset with no spontaneous diuresis. |
| Ht. 96.0 cm. | 12/16–21 | Cortisone 335 mg. (6d) | 24.7–25.2 | — | — | — | — | — | — | — | — | — | | | | |
| Wt. 15.2 kg.‡ | 1/4/50 | | 21.4 | — | — | — | — | — | — | — | — | — | | | | |
| S.A. 0.62M$^2$ | 1/4–16 | ACTH 1000 mg. (13d)† | 21.4–20.9 | — | — | — | — | — | — | — | — | — | | | | |
| | 1/19 | | 20.3 | — | — | — | — | — | — | — | — | — | | | | ‖Diuresis 15th day after start of therapy (1/19). |
| | 1/30 | | 14.2 | — | — | — | — | — | — | — | — | — | | | | Minimum weight following diuresis. |
| | 2/1 | | 14.6 | 14.3 | 19.9 | 12.8 | — | — | — | 1.4 | 0.9 | — | | | | Outset of reaccumulation of edema. |
| | 2/16 | | 19.6 | — | — | — | — | — | — | — | — | — | | | | Maximum weight. |
| | 2/23 | | 19.1 | — | — | — | — | — | — | — | — | — | | | | |
| | 2/24–27 | ACTH 350 mg. (3½d) | 18.5–19.1 | — | — | — | — | — | — | — | — | — | | | | No diuresis. |
| | 2/28 | | 19.3 | 10.1 | 18.6 | — | 70.9 | — | 0.14 | 1.8 | — | — | | | | |
| | 3/7 | | 19.6 | — | — | — | — | — | — | — | — | — | 663 | 1074 | 40.0 | |
| | 3/8–17 | ACTH 950 mg. (9½d) | 19.6–21.4 | — | — | — | — | — | — | — | — | — | | | | |
| | 3/21 | | 20.9 | 28.2 | 30.4 | — | 119 | — | 0.24 | 1.1 | — | — | | | | Diuresis 13th day after start of therapy (3/21) |
| | 3/22 | | 20.0 | — | — | — | — | — | — | — | — | — | 853 | 1218 | 31.4 | |
| | 3/27 | | 15.0 | — | — | — | — | — | — | — | — | — | | | | Minimum weight following diuresis. |
| | 3/30 | | 15.4 | — | — | — | — | — | — | — | — | — | | | | Outset of reaccumulation of edema. |
| | 5/10 | | 17.5 | — | — | — | — | — | — | — | — | — | | | | |
| | 5/11–24 | ACTH 687.5 mg. (14d) | 17.2–20.7 | — | — | — | — | — | — | — | — | — | | | | |
| | 5/24 | | 20.7 | 38.2 | 37.5 | 33.2 | 106 | — | 0.36 | 1.0 | — | — | | | | |
| | 5/26 | | 20.1 | — | — | — | — | — | — | — | — | — | | | | Diuresis 15th day after start of therapy (5/26). |
| | 11/16 | | 17.5 | — | — | — | — | — | — | — | — | — | | | | In apparent clinical remission 12/1/50. |
| Expected normal value § | | | | *44.1* | | | *225* | | *0.20* | | | | | | | |
| K. N. | 1/20/50 | | 20.5 | 8.1 | 16.6 | — | 71.9 | — | 0.11 | 2.0 | — | — | | | | Duration of disease—10 mos. |
| M. Age 3 yrs. | 1/20–27 | ACTH 675 mg. (8d) | 20.5–20.6 | — | — | — | — | — | — | — | — | — | | | | Constant edema since outset with no spontaneous diuresis. |
| Ht. 91.5 cm. | 1/29 | | 20.3 | — | — | — | — | — | — | — | — | — | | | | Diuresis 9th day after start of therapy (1/29). |
| Wt. 14.1 kg. | 2/3 | | 14.3 | 25.2 | 30.1 | — | 94.2 | — | 0.27 | 1.2 | — | — | | | | |
| S.A. 0.58M$^2$ | 2/6 | | 12.2 | — | — | — | — | — | — | — | — | — | | | | Minimum weight following diuresis. |
| | 2/9 | | 12.5 | — | — | — | — | — | — | — | — | — | | | | Outset of reaccumulation of edema. |
| | 3/3 | | 18.4 | — | — | — | — | — | — | — | — | — | 553 | 819 | 34.0 | Maximum weight. |
| | 3/4–13 | ACTH 950 mg. (9½d) | 18.3–17.4 | — | — | — | — | — | — | — | — | — | | | | |
| | 3/10 | | 19.7 | — | — | — | — | — | — | — | — | — | | | | Diuresis 6th day after start of therapy (3/10). |
| | 3/11 | | 19.1 | — | — | — | — | — | — | — | — | — | | | | |
| | 3/13 | | 17.4 | — | — | — | — | — | — | — | — | — | 645 | 904 | 30.0 | |
| | 3/18 | | 12.4 | — | — | — | — | — | — | — | — | — | | | | Minimum weight following diuresis. |
| | 3/21 | | 12.9 | — | — | — | — | — | — | — | — | — | | | | Outset of reaccumulation. |
| | 4/20 | | 15.9 | — | — | — | — | — | — | — | — | — | | | | |
| | 4/21 | | 16.2 | — | — | — | — | — | — | — | — | — | | | | Maximum weight. |
| | 4/21–5/1 | ACTH 537.5 mg. (11d) | 16.2–15.4 | — | — | — | — | — | — | — | — | — | | | | Diuresis 8th day after start of therapy (4/29). |
| | 4/29 | | 17.2 | — | — | — | — | — | — | — | — | — | | | | |
| | 4/30 | | 16.6 | — | — | — | — | — | — | — | — | — | | | | |
| | 4/31 | | 16.1 | — | — | — | — | — | — | — | — | — | | | | |
| | 5/4 | | 13.9 | — | — | — | — | — | — | — | — | — | | | | Minimum weight following diuresis. |
| | 5/31 | | 16.1 | 27.6 | 38.2 | — | 79.8 | — | 0.35 | 1.4 | — | — | | | | |
| | 11/17 | | 15.5 | — | — | — | — | — | — | — | — | — | | | | In apparent clinical remission 12/1/50. |
| Expected normal value | | | | *41.2* | | | *211* | | *0.20* | | | | | | | No manifest edema. |

* Each value represents the average of three or more 10 to 20 minute clearance periods.

† Daily dosage of ACTH was given in four divided doses at six hour intervals.

‡ Ideal weight for height and age (13) was used for the estimation of surface area (14) from which expected normal values for renal plasma clearances were calculated.

§ These values were calculated as follows:

$$\text{Expected normal value} = \frac{\text{Surface area of child (M}^2\text{)}}{1.73\text{M}^2} \times \text{normal adult value corrected to } 1.73\text{M}^2.$$

Since there is no evidence that a sex difference in renal functions exists in children before puberty, a single normal adult value for each discrete function was used to calculate expected normal values for both boys and girls. These values were arbitrarily taken as the average of mean values for normal men and women (15). Per unit of surface area, values for discrete kidney functions for children of this age are expected to be within the normal adult range (16).

‖ Day on which weight loss of 0.2 kg. or greater was first observed and which was followed by continued loss of body weight.

TABLE I—*Continued*

| Subject | Date | Hormone administration | Weight | $C_{IN}$ | $C_{CR}$ | $C_{THIO}$ | $C_{PAH}$ | $T_{M_{PAH}}$ | $C_{IN}/C_{PAH}$ | $C_{CR}/C_{IN}$ | $C_{THIO}/C_{IN}$ | $C_{IN}/T_{M_{PAH}}$ | Plasma volume | Blood volume | Hematocrit | Comment |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | *kg.* | *ml. per min.* | *ml. per min.* | *ml. per min.* | *ml. per min.* | *mg. per min.* | | | | | *ml.* | *ml.* | *%* | |
| B. B. | 3/16/50 | | 18.3 | 14.0 | 28.1 | 16.2 | 148 | — | 0.09 | 2.0 | 1.2 | — | | — | | Duration of disease—7 mos. |
| Age 3.5 yrs. | 3/17 | | 19.0 | — | — | — | — | — | — | — | — | — | 615 | 814 | 25.6 | Constant edema since outset with no spontaneous diuresis. |
| t. 90.0 cm. | 3/17–26 | ACTH 740 mg. (*9d*) | 19.0–19.8 | — | — | — | — | — | — | — | — | — | | | | |
| t. 13.7 kg. | 3/28 | | 19.3 | — | — | — | — | — | — | — | — | — | | | | Diuresis 11th day after start of therapy (3/28). |
| A. 0.57$M^2$ | 3/29 | | 17.7 | — | — | — | — | — | — | — | — | — | 774 | | | |
| | 3/31 | | 14.8 | 40.6 | 44.5 | 39.0 | 197 | — | 0.21 | 1.1 | 1.0 | — | | | | |
| | 4/3 | | 12.4 | — | — | — | — | — | — | — | — | — | | | | Minimum weight following diuresis. |
| | 4/20 | | 18.6 | — | — | — | — | — | — | — | — | — | | | | Maximum weight. |
| | 4/21 | | 18.4 | — | — | — | — | — | — | — | — | — | | | | |
| | 4/21–5/15 | ACTH 893.8 mg. (25d) | 18.4–15.9 | — | — | — | — | — | — | — | — | — | | | | |
| | 5/1 | | 19.1 | — | — | — | — | — | — | — | — | — | | | | Diuresis 10th day after start of therapy (5/1). |
| | 5/7 | | 13.6 | — | — | — | — | — | — | — | — | — | | | | Minimum weight following therapy. |
| | 5/9 | | 14.1 | — | — | — | — | — | — | — | — | — | | | | In apparent clinical remission 5/9–10/1. |
| | 6/12 | | 16.5 | 54.0 | 55.7 | — | 211 | — | 0.26 | 1.0 | — | — | | | | Reaccumulation of edema 10/1/50. |
| | 11/2 | | 18.2 | — | — | — | — | — | — | — | — | — | | | | 13 days ACTH therapy (525 mg.) resulted in diuresis. |
| Expected normal value | | | | *40.5* | | | *207* | | *0.20* | | | | | | | |
| J. S. | 3/6/50 | | 13.9 | 41.2 | 57.9 | 46.3 | 196 | — | 0.21 | 1.4 | 1.1 | — | | | | Duration of disease—2.5 mos. |
| Age 3.2 yrs. | 4/11 | | 15.3 | 45.8 | 58.6 | — | *196* | — | 0.23 | 1.3 | — | — | | | | *One spontaneous* diuresis 4 weeks after outset. |
| t. 95.0 cm. | 4/12 | | — | — | — | — | — | — | — | — | — | — | 678 | 1064 | 38.0 | Minimal edema at start of therapy. |
| t. 15.0 kg. | 4/14–20 | ACTH 625 mg. (6½d) | 15.6–15.9 | — | — | — | — | — | — | — | — | — | | | | |
| A. 0.61$M^2$ | 4/21 | | 15.7 | — | — | — | — | — | — | — | — | — | 750 | 1143 | 36.0 | |
| | 4/22 | | 15.2 | 42.8 | 46.4 | — | 193 | — | 0.22 | 1.1 | — | — | | | | Diuresis 8th day after start of therapy (4/22). |
| | 4/24 | | 14.7 | — | — | — | — | — | — | — | — | — | | | | Minimum weight following diuresis. |
| | 8/31 | | 18.0 | — | — | — | — | — | — | — | — | — | | | | |
| Expected normal value | | | | *45.5* | | | *221* | | *0.20* | | | | | | | Moderate intermittent peripheral edema 12/1/50. |
| R. K. | | | | | | | | | | | | | | | | |
| Age 3.9 yrs. | 7/10/50 | | 27.4 | 37.1 | 48.1 | — | 212 | — | 0.18 | 1.3 | — | — | | | | Duration of disease—11 mos. |
| t. 100 cm. | 7/11 | | 27.8 | — | — | — | — | — | — | — | — | — | 933 | 1510 | 40.0 | One diuresis in March 1950 following paracentesis<br>Reaccumulation of edema 4 months later. |
| t. 16.1 kg. | 7/12 | | 27.6 | — | — | — | — | — | — | — | — | — | | | | |
| A. 0.64$M^2$ | 7/12–22 | ACTH 500 mg. (11d) | 27.6–27.8 | — | — | — | — | — | — | — | — | — | | | | |
| | 7/23 | | 27.2 | — | — | — | — | — | — | — | — | — | | | | Diuresis 11th day after start of therapy (7/23). |
| | 7/27 | | 19.3 | 56.0 | 64.5 | — | 233 | — | 0.24 | 1.2 | — | — | 918 | 1463 | 39.0 | |
| | 8/1 | | 17.2 | — | — | — | — | — | — | — | — | — | | | | Minimum weight following diuresis. |
| | 11/26 | | 21.8 | — | — | — | — | — | — | — | — | — | | | | Reaccumulation of edema and spontaneous diuresi in October and again in November. |
| Expected normal value | | | | *45.5* | | | *233* | | *0.20* | | | | | | | |

TABLE I—*Continued*

*Children's Hospital of Philadelphia*

| Subject | Date | Hormone administration | Weight | $C_{IN}$ | $C_{CR}$ | $C_{THIO}$ | $C_{PAH}$ | $T_{M_{PAH}}$ | $C_{IN}/C_{PAH}$ | $C_{CR}/C_{IN}$ | $C_{THIO}/C_{IN}$ | $C_{IN}/T_{M_{PAH}}$ | Plasma volume | Blood volume | Hematocrit | Comment |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | *kg.* | *ml. per min.* | *ml. per min.* | *ml. per min.* | *ml. per min.* | *mg. per min.* | | | | | *ml.* | *ml.* | % | |
| R. T. | 1/4/50 | | 17.5 | 49.4 | — | — | 160 | 31.3 | 0.31 | — | — | 1.6 | | | | Duration of disease—4 mos. |
| M. Age 3 yrs. | 1/6–22 | Cortisone 1300 mg. (17d) | — | — | — | — | — | — | — | — | — | — | | | | Constant edema since outset with no spontaneous diuresis. |
| Ht. 91.5 cm. | 1/16 | | 20.0 | 45.1 | — | — | 220 | 39.0 | 0.21 | — | — | 1.2 | | | | No diuresis following Cortisone. |
| Wt. 14.1 kg. | 2/20 | | 16.8 | 37.5 | — | — | 123 | 34.7 | 0.30 | — | — | 1.1 | | | | |
| S.A. 0.58M² | 3/1–8 | Cortisone 575 mg. (8d) | | — | — | — | — | — | — | — | — | — | | | | No diuresis. |
| | | DCA 40 mg. | | — | — | — | — | — | — | — | — | — | | | | |
| | 3/29 | | 22.5 | — | — | — | — | — | — | — | — | — | | | | |
| | 3/29–4/8 | ACTH 880 mg. (11d) | | — | — | — | — | — | — | — | — | — | | | | |
| | 4/10 | | 22.5 | 44.1 | — | — | 200 | 27.9 | 0.22 | — | — | 1.6 | | | | |
| | 4/11 | | 22.5 | — | — | — | — | — | — | — | — | — | | | | Diuresis 13th day after start of therapy (4/11). |
| | 4/14 | | 20.5 | 46.0 | — | — | 168 | 25.7 | 0.27 | — | — | 1.8 | | | | Minimum weight following diuresis. |
| | 4/15 | | 15.0 | — | — | — | — | — | — | — | — | — | | | | |
| | 5/8 | | 17.0 | 43.5 | — | — | 154 | 29.4 | 0.28 | — | — | 1.5 | | | | A second course of ACTH therapy (5/22–6/6 920 mg.) induced no diuresis. |
| | 5/26 | | 22.3 | — | — | — | — | — | — | — | — | — | | | | |
| | 8/7 | | 25.5 | — | — | — | — | — | — | — | — | — | | | | Generalized edema continues 8/17/50. |
| Expected normal value | | | | *41.2* | | | *211* | *25.9* | *0.20* | | | *1.6* | | | | |
| M. L. | 3/2/50 | | 20.7 | 29.5 | — | — | 170 | 20.7 | 0.17 | — | — | 1.4 | | | | Duration of disease—1 mo. |
| M. Age 3 yrs. | 3/4 | | 21.4 | — | — | — | — | — | — | — | — | — | | | | Constant edema with no spontaneous diuresis. |
| Ht. 91.5 cm. | 3/4–13 | ACTH 800 mg. (10d) | — | — | — | — | — | — | — | — | — | — | | | | |
| Wt. 14.1 kg. | 3/14 | | 23.9 | 44.2 | — | — | 198 | 28.2 | 0.22 | — | — | 1.6 | | | | |
| S.A. 0.58M² | 3/15 | | 21.4 | — | — | — | — | — | — | — | — | — | | | | Diuresis 11th day after start of therapy (3/15). |
| | 3/20 | | 15.9 | 43.1 | — | — | 157 | 24.7 | 0.27 | — | — | 1.7 | | | | |
| | 3/22 | | 14.5 | — | — | — | — | — | — | — | — | — | | | | Minimum weight following diuresis. |
| Expected normal value | | | | *41.2* | | | *211* | *25.9* | *0.20* | | | *1.6* | | | | No manifest edema—8/17/50. |
| S. S. | 2/27/50 | | 15.0 | 29.0 | — | — | 241 | 16.0 | 0.12 | — | — | 1.8 | | | | Duration of disease—15 mos. |
| M. Age 3.5 yrs. | 3/2–8 | Cortisone 400 mg. (7d) | — | — | — | — | — | — | — | — | — | — | | | | Constant edema since outset with no spontaneous diuresis. |
| Ht. 94.0 cm. | 3/9 | | 19.3 | 41.3 | — | — | 193 | 14.0 | 0.21 | — | — | 3.0 | | | | |
| Wt. 14.5 kg. | 3/23 | | 15.0 | 17.1 | — | — | 129 | 12.0 | 0.13 | — | — | 1.4 | | | | |
| S.A. 0.60M² | 3/27 | | 15.0 | — | — | — | — | — | — | — | — | — | | | | No diuresis after Cortisone. |
| | 3/27–4/8 | ACTH 1040 mg. (13d) | — | — | — | — | — | — | — | — | — | — | | | | |
| | 4/4 | | 15.6 | — | — | — | — | — | — | — | — | — | | | | |
| | 4/6 | | 15.6 | 60.8 | — | — | 238 | 17.7 | 0.26 | — | — | 3.4 | | | | Diuresis 8th day after start of therapy (4/4). |
| | 4/9 | | 12.7 | — | — | — | — | — | — | — | — | — | | | | |
| | 4/11 | | 13.2 | 34.3 | — | — | 211 | 26.1 | 0.16 | — | — | 1.3 | | | | Minimum weight. |
| Expected normal value | | | | *42.7* | | | *218* | *26.8* | *0.20* | | | *1.6* | | | | No manifest edema 8/17/50. |

in the $C_{IN}:C_{PAH}$ ratio are associated with vascular changes or alterations in the permeability of the glomerular membrane cannot be assessed at present.

### *Maximum tubular excretion of PAH*

Increases in $Tm_{PAH}$ accompanied increases in $C_{IN}$ in two children (M. L. and S. S.) in whom it was measured before and during diuresis. The changes in $Tm_{PAH}$ were less than those in $C_{IN}$ so that the $C_{IN}:Tm_{PAH}$ ratios increased from initially low values before to higher values. In the remaining child in whom repeated measurements of $Tm_{PAH}$ were made, no change in $C_{IN}$ was observed and the changes in $Tm_{PAH}$ were irregular.

### *Plasma and blood volume*

Increases in plasma volume during or after diuresis were observed in four out of five children in whom such observations were made. The changes in I. C., K. N., B. B., and J. S. were 29, 17, 26, and 11% respectively, above the control values. Corresponding increases in blood volume in I. C., K. N., and J. S. were observed but the changes were proportionately less because of decreases in hematocrit values. Failure to observe an increase in plasma volume in R. K. may possibly be explained again by a failure to make the measurement at the proper time.

## DISCUSSION

Prompt increases above initially reduced rates of glomerular filtration are commonly seen during the early stages of acute nephritis in children. On the other hand, reduced clearances in children with the nephrotic syndrome of several months' duration have been interpreted as indicating a less reversible process. The rapid increases in glomerular filtration rate reported here in children who had reduced kidney function and who were given ACTH demonstrate, therefore, a potentiality for improvement in function beyond that commonly considered possible in children with the nephrotic syndrome.

There is no evidence and it is not implied that these results are peculiar to diureses induced by ACTH. Although the magnitude of the changes was somewhat greater than those previously reported with other agents (1, 4, 19), it is entirely possible and probable that quantitatively similar changes may accompany spontaneous or induced diureses.

The results described have no bearing on the question of whether pituitary or adrenal cortical hormones have any direct relationship to the nephrotic syndrome. They do indicate that ACTH administration may induce repeated diureses at predictable times permitting serial measurements of changes which may accompany and be involved in the mechanism of diuresis. Thus large increases in glomerular filtration rate and significant increases in plasma volume were observed during diureses in these children. These observations lend support to the concept (20) that increases in plasma volume and in glomerular filtration rate accompany and may be involved in the mechanism of diuresis in children with the nephrotic syndrome.

## SUMMARY

Profuse, sustained diureses occurring at predictable times in a high percentage of trials during or following administration of ACTH to children with the nephrotic syndrome permitted observations on changes which may accompany and be involved in the mechanism of diuresis. Serial measurements of discrete kidney functions and plasma volume are reported before, during and after 13 such diureses in eight children. The results indicate:

1. In five children with initially reduced rates of glomerular filtration, increases in inulin clearances ranging from 50 to 256% above control values were observed during or following diureses on five out of six occasions. In three observations on three children with rates of glomerular filtration initially within the normal range, an increase of 54% was observed in one. Thus in nine observations where inulin clearances were measured before and after the outset of diuresis, significant increases were observed in six.

2. Daily 24-hour creatinine clearances, measured in one of the three instances in which no marked increase in inulin clearance was observed *after* diuresis, suggest that glomerular filtration rate did increase *during* diuresis. In addition, the 24-hour clearances suggest that a marked increase in glomerular filtration rate during diuresis may be followed by a decrease from the maximum

value, but with subsequent stabilization at a value slightly or markedly above the control. Repeated responses in children with reduced function may then be associated with a progressive increase to normal or even "supernormal" values.

3. A consistent decrease in the endogenous creatinine: inulin clearance ratio was observed as glomerular filtration rate increased. However, clearances of thiosulfate and inulin showed reasonably good agreement at both high and low rates of glomerular filtration.

4. Clearances and maximum tubular excretion of p-aminohippurate increased during diuresis but proportionately less than inulin clearances. Consequently, increases in the $C_{IN}:C_{PAH}$ and the $C_{IN}:Tm_{PAH}$ ratios were observed as rates of glomerular filtration increased.

5. In four of five observations on five children, increases in plasma volume (T-1824) ranging from 11 to 29% above control values were observed during diureses.

### CONCLUSIONS

1. Diureses occurring during or following administration of ACTH to children with the nephrotic syndrome are usually associated with marked improvement in kidney function. Repeated diureses in children with initially reduced function may be associated with progressive increases in rates of glomerular filtration to normal values. These observations demonstrate a potentiality for improvement in function beyond that commonly considered possible in the nephrotic syndrome of many months' duration. It is believed that these changes are not peculiar to diureses associated with ACTH administration but may accompany spontaneous or other types of induced diureses.

2. The changes observed in plasma volume and glomerular filtration rate support the concept that increases in plasma volume and glomerular filtration rate are associated with the mechanism of diuresis in children with the nephrotic syndrome.

### ACKNOWLEDGMENTS

We are indebted to Flora Hurwitz, R.N., Marie Ragni, B.A., Jean Tracy, R.N., and Josephine Codis, R. N., for nursing and technical assistance.

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# THE EFFECTS OF ADRENOCORTICOTROPIC HORMONE AND CORTISONE IN THE ADRENOGENITAL SYNDROME ASSOCIATED WITH CONGENITAL ADRENAL HYPERPLASIA: AN ATTEMPT TO EXPLAIN AND CORRECT ITS DISORDERED HORMONAL PATTERN [1, 2]

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(Submitted for publication November 2, 1950; accepted, December 9, 1950)

The adrenogenital syndrome associated with congenital adrenal hyperplasia (1) is in many of its aspects the direct opposite of the other syndrome which may result from adrenal hyperplasia, namely, Cushing's syndrome (2). For example, the former is characterized by rapid growth before epiphyseal closure, and an increase in strength, muscle mass, and skin thickness, whereas the latter is characterized by cessation of growth before epiphyseal closure, and a decrease in strength, muscle mass and skin thickness.

Since the effect of adrenocorticotropic hormone (ACTH) in subjects with normal adrenals is essentially to produce a temporary "Cushing's syndrome medicamentosa" (3–7) it was of interest to observe the effects of ACTH in the adrenogenital syndrome. The first part of this report concerns the effects of ACTH in three female patients with the adrenogenital syndrome.

The results, which were surprising, led to the supposition that cortisone might inhibit the excessive production of androgens in these patients. The second part of this report concerns the effects of cortisone in two female patients with the adrenogenital syndrome.

## METHOD

With the exception of the long-term studies which are still in progress (*vide infra*) all studies were carried out on the Metabolic Ward. The diet and fluid intake were kept constant throughout each study. Stools were collected in six day periods, and urines in one to six day pools. Stools and diets were analyzed for N, P, Ca, K, Na, and Mg,[5] and urines for these constituents and for Cl, "reducing steroids," and 17-ketosteroids. Fasting blood for determination of circulating eosinophils, and of serum glucose, Ca, P, Mg,[5] Na, K, Cl, and $CO_2$ was drawn at appropriate intervals. The following methods were used: Magnesium—Briggs (8); reducing steroids—Talbot and his associates (9) and Heard, Sobel and Venning (10); eosinophil counts— Hills, Forsham and Finch (11), except that the blood was diluted 1:10 instead of 1:20; the remaining determinations—as described elsewhere (12).

## CASE HISTORIES

C. O., M.G.H. No. 169, 536, was a 13 year old girl who had been seen first at the age of five because of the presence of pubic hair since the age of two. Growth proceeded rapidly, with markedly advanced bone age, and ceased by the age of nine with epiphyseal closure. Menses had not appeared.

Physical examination revealed an abnormally muscular girl, a height and span both of 57½ inches, a large thyroid cartilage, a deep voice, temporal recession of the hairline, hirsutism of face and body, a large amount of pubic and axillary hair, scant breast tissue, a very large clitoris, absent labia minora, and a common external orifice of urethra and vagina. X-rays revealed no adrenal tumor after air insufflation, and a normal skull. Repeated chemical analyses revealed normal serum glucose, Na, Cl, K, Ca, P, $CO_2$ content, and alkaline phosphatase. A Cutler-Power-Wilder test revealed a normal four hour chloride excretion. Twenty-four hour urine specimens contained normal amounts of reducing steroids, subnormal amounts (less than 2.8 mouse units) of "cortin" (13), and very large amounts (60 to 70 mg.) of 17-ketosteroids.

An electroencephalogram was read by Dr. John Abbott as follows: "This is an abnormal record for her age. It shows a mild, diffuse, slow-wave cerebral dysrhythmia (many six per second waves). Voltages are slightly higher on the right."

T. McH., M.G.H. No. 586, 528, was a ten year old girl

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[1] Reported in part at the Proceedings of American Society for Clinical Investigation, May 1, 1950. (J. Clin. Invest., 1950, 29, 797.)

[2] The expense of these studies was defrayed by a grant from the Rockefeller Foundation, a grant from Ayerst, McKenna and Harrison, Ltd., and grants from the American Cancer Society. A bed supported by Mr. Edward Mallinckrodt, Jr., on the Metabolic Ward was used for these studies.

[3] Surgeon, U.S.P.H.S., National Heart Institute.

[4] National Research Council Fellow in the Medical Sciences, 1949–1951.

[5] Not done on patient C. O.

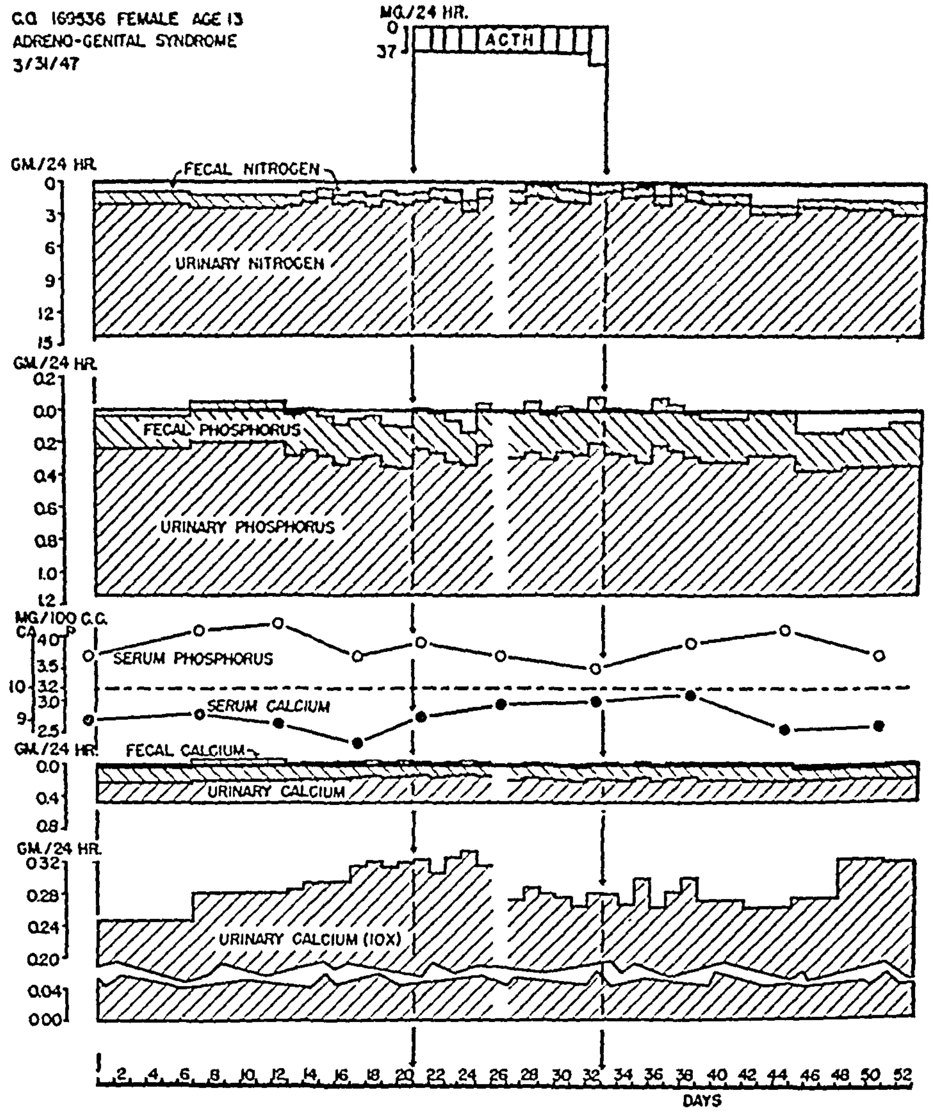

Fig. 1. N, P, and Ca Balances, Urinary Ca, and Serum P and Ca during Administration of ACTH to C. O., a 13 Year Old Female with the Adrenogenital Syndrome

Balance data in this and subsequent charts are plotted as follows: There is a horizontal base line; intake is charted downward from this base line; the urinary and fecal excretions are then measured upward from the intake line towards the base line. If the output (fecal and urinary) exceeds the intake, the final level will be above the base line; if it does not, the final level will be below the base line. Thus a negative balance is indicated by a shaded area above the base line, and a positive balance by a clear area below the base line. The scales for N, P, Ca, and K metabolism are so chosen that (for changes in protoplasm and bone) the area representing P balance should equal the sum of the corresponding areas for N and Ca; and that representing K balance should equal that for N.

who was referred to us by Dr. Gertrud Ryersbach. She had been seen first at the age of eight because of the presence of pubic hair since the age of four. Growth proceeded rapidly, with markedly advanced bone age. Menses had not appeared except upon withdrawal of estrin therapy, which had been tried without appreciable effect on the virilism.

Physical examination revealed a well-developed girl, a height and span of 65 and 65¾ inches respectively, a slightly enlarged thyroid cartilage, a voice not deeper than that of a normal adult female, a large amount of pubic and a moderate amount of axillary hair, a moderate amount of breast tissue (estrogen therapy), a very large clitoris, and otherwise normal external genitalia. X-rays revealed a normal skull. Repeated chemical analyses revealed normal serum glucose, Na, Cl, K, Ca, P, $CO_2$ content, and alkaline phosphatase. A Kepler water test gave a normal result. Twenty-four hour urine specimens contained normal amounts of reducing steroids, and large amounts (14 to 18 mg.) of 17-ketosteroids.

Ten electroencephalograms were read by Dr. John Abbott as follows: "These are abnormal records for her age. They show severe diffuse, slow-wave cerebral dysrhythmia (random waves and paroxysmal runs, notably three per second waves reaching 150 micro-volts, and six per second waves reaching 175 micro-volts). Voltages are markedly higher, and dysrhythmia is more pronounced, on the right. These abnormalities are especially

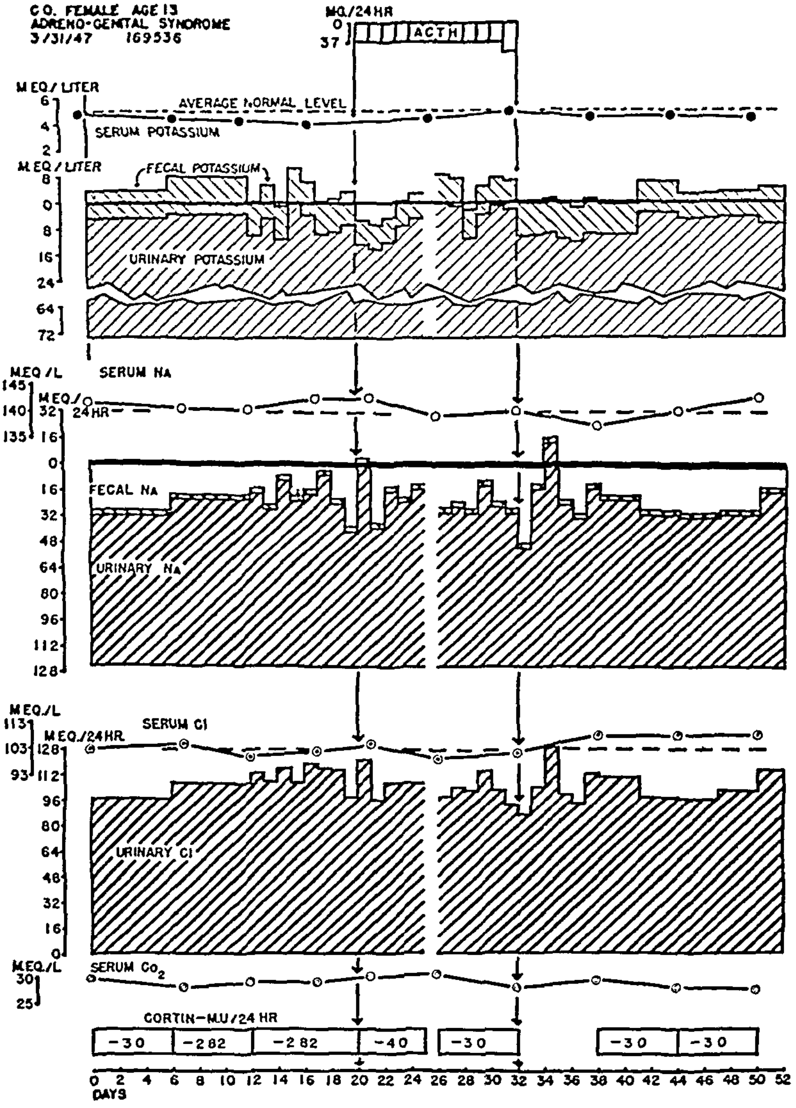

FIG. 2. K AND NA BALANCES, URINARY CL AND CORTIN, AND SERUM K, NA, CL AND $CO_2$ CONTENT IN EXPERIMENT SHOWN IN FIGURE 1

For interpretation of cortin see Forbes, Griswold and Albright (25).

marked in the occipitoparietal region a little to the right of the midline. There are traces of normal alpha activity at eight per second and 50 micro-volts."

On explorations by Doctor Oliver Cope, the internal genitalia were found to be of the normal female structure; ovarian biopsy revealed developing follicles; the right adrenal gland was not grossly abnormal to palpation; and the left adrenal cortex showed hypertrophy on biopsy.

R. H., M.G.H. No. 401, 611, was a 12 year old girl who had been seen first at the age of five because of the presence of an enlarged clitoris since the age of three months and pubic hair since the age of four. Growth proceeded rapidly, with advanced bone age, and ceased by the age of nine with epiphyseal closure. Menses had not appeared, even on withdrawal of estrogen therapy, which had been tried without appreciable effect on the virilism.

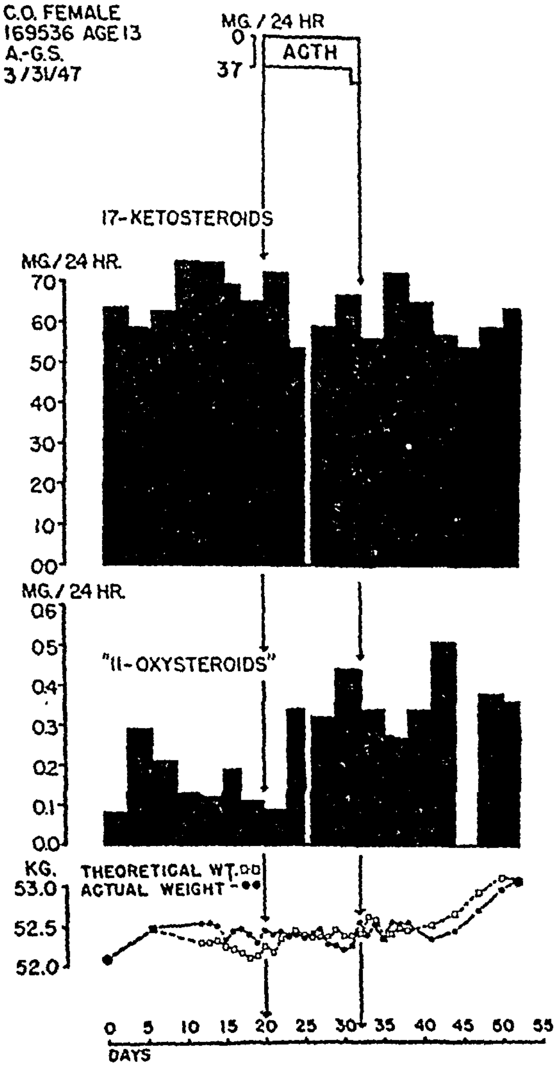

FIG. 3. 17-KETOSTEROIDS AND "11-OXYCORTICOSTEROIDS," ACTUAL WEIGHT, AND THEORETICAL WEIGHT BASED ON N, NA, AND K DURING EXPERIMENT SHOWN IN FIGURE 1

For calculation of theoretical weight see Reifenstein and associates (12). The upper limit of normal for "11-oxycorticosteroids" by this method is 0.44 mg. per 24 hours (26).

Physical examination revealed a well-developed girl, a height and span both of 62 inches, a slightly enlarged thyroid cartilage, a moderately deep voice, slight temporal recession of the hairline, hirsutism of the upper lip and body, large amounts of pubic and axillary hair, a moderate amount of breast tissue (estrogen therapy), a very large clitoris, absent labia minora, and a common external orifice of urethra and vagina. X-rays revealed normally placed kidneys and a normal skull. Repeated chemical analyses revealed normal serum glucose, Na, Cl, K, Ca, P, $CO_2$ content, and alkaline phosphatase. Twenty-four hour urine specimens contained normal amounts of reducing steroids and large amounts (17 to 25 mg.) of 17-ketosteroids.

Four electroencephalograms were read by Dr. John Abbott as follows: "These are abnormal records for her age. They show a diffuse, slow-wave, cerebral dysrhythmia (four to six per second waves). The abnormality is somewhat more marked on the left. There is some normal alpha activity at around eight to nine per second and 40 micro-volts."

## RESULTS

The overall design of each metabolic study may be seen by inspection of Figures 1, 4, and 7. Seven courses of therapy were given as shown in Table I. The metabolic effects of the two hormones are best considered separately.

### I. ACTH

#### a) Nitrogen metabolism

ACTH did not produce a significantly negative nitrogen balance in any of the four courses of administration (Figures 1, 4, and 7). Indeed, when a daily dose of 200 mg. was given, *there was actual nitrogen retention.* This observation is contrary to the experience of ourselves (14, 15) and others (3–6) in patients with essentially normal adrenal cortical function. Figure 10 is a scatter diagram contrasting the total six day N excretion of other patients given comparable doses of ACTH with that of these patients.

#### b) Phosphorus metabolism

When 200 mg. of ACTH were given to patient T. McH. (Figure 4) there was retention of P commensurate with that of N, on the assumption that protoplasm was formed (12). In the other experiments (Figures 1 and 7) ACTH did not affect P balance or serum P.

TABLE I

*The dosage and duration of ACTH and cortisone administration and the composition of the diet in the metabolic studies reported*

| Wt. at start of treatment | Patient | Drug | Daily dose | | Units pressor per day† | No. of days | CHO | Fat | Protein | Calories |
|---|---|---|---|---|---|---|---|---|---|---|
| *kg.* | | | *mg.** | *mg.* per kg.* | | | | | | |
| 52 | C. O. | ACTH | 37 | .71 | 2.6 | 12‡ | 250 | 103 | 83 | 2258 |
| 55 | T. McH. | ACTH§ | 84 | 1.53 | 4 | 12 | 246 | 61 | 74 | 1828 |
| 60 | T. McH. | ACTH | 200 | 3.33 | 8 | 12 | 388 | 57 | 59 | 2317 |
| 47 | R. H. | ACTH | 84 | 1.79 | 4 | 9 | 179 | 57 | 54 | 1446 |
| 53 | T. McH. | Cortisone acetate§ | 100 | 1.90 | | 12 | 246 | 61 | 74 | 1828 |
| 45 | R. H. | Cortisone acetate | 100 | 2.22 | | 12 | 179 | 57 | 54 | 1446 |
| 43 | R. H. | Combined ACTH and cortisone | 88<br>100 | 2.05<br>2.32 | 4 | 8 | 179 | 57 | 54 | 1446 |

* Expressed as equivalents of Armour Standard La 1A.

† The pressor is present in the ACTH as contaminant and was assayed by the Armour Laboratories.

‡ On the 12th day, 55 mg., or 1.06 mg. per kg., were given.

§ Stilbestrol, 5 mg. a day, was administered throughout this experiment.

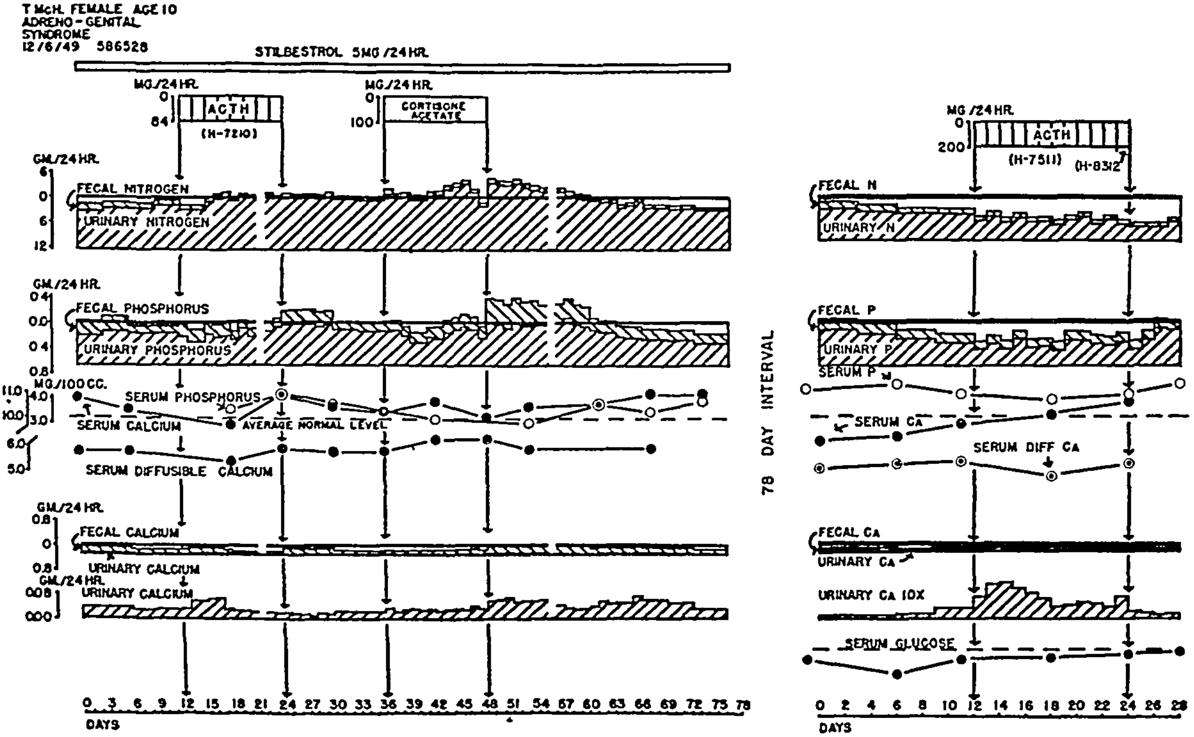

FIG. 4. N, P, AND CA BALANCES, URINARY CA, SERUM P, CA AND DIFFUSIBLE CA DURING ADMINISTRATION OF ACTH AND CORTISONE TO T. MCH., A TEN YEAR OLD FEMALE WITH THE ADRENOGENITAL SYNDROME

The determinations of diffusible Ca in this and the subsequent experiments were done for us by Dr. John Dawson.

*c) Calcium metabolism*

There was a rise in urinary calcium with ACTH in all experiments save that on C.O. (Figure 1). As discussed elsewhere (7, 16) this effect may be due to the pitressin present in the ACTH as a contaminant (Table I). Comparison of the actual weight curves with the "theoretical" weight curves based on N-plus-K-plus Na (Figures 3, 6, and 9) shows that water was retained without electrolytes during ACTH therapy—presumably a pitressin effect—in the experiments on T. McH. and R. H. Water was not retained in C. O., and she showed no rise in urinary calcium.

*d) Potassium metabolism*

ACTH produced a slight, unsustained retention of K in all experiments. This is in contrast to our previous experience (7), wherein a transitory rise of urinary K, with negative balance, on the first day of therapy, and a transitory fall, with positive balance, on the first or second day after stopping have been among the most constant effects of ACTH. This is shown in Figure 11, wherein the effect of ACTH on urinary K excretion in 12 experiments on patients with essentially normal adrenal cortical function is contrasted with that in these patients.

*e) Sodium and chloride metabolism*

In the first experiment on T. McH. and that on C. O., ACTH had no appreciable effect on Na or Cl metabolism (Figures 2 and 5). In the other two experiments, slight loss of Na and Cl occurred (Figures 5 and 8). As discussed elsewhere, the effect of these preparations of ACTH upon Na and Cl appears to be a resultant of the

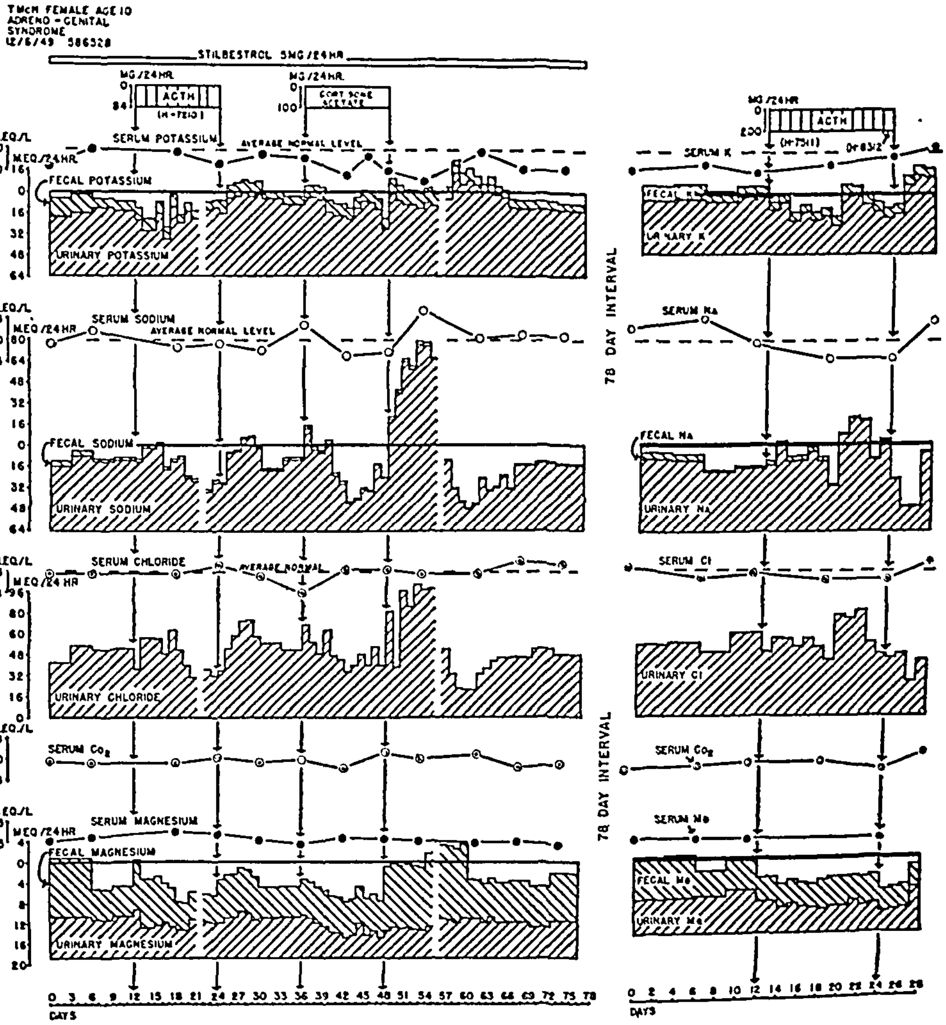

Fig. 5. K, Na and Mg Balances, Urinary Cl and Serum K, Na, Cl, CO₂ Content, and Mg during the Experiment Shown in Figure 4

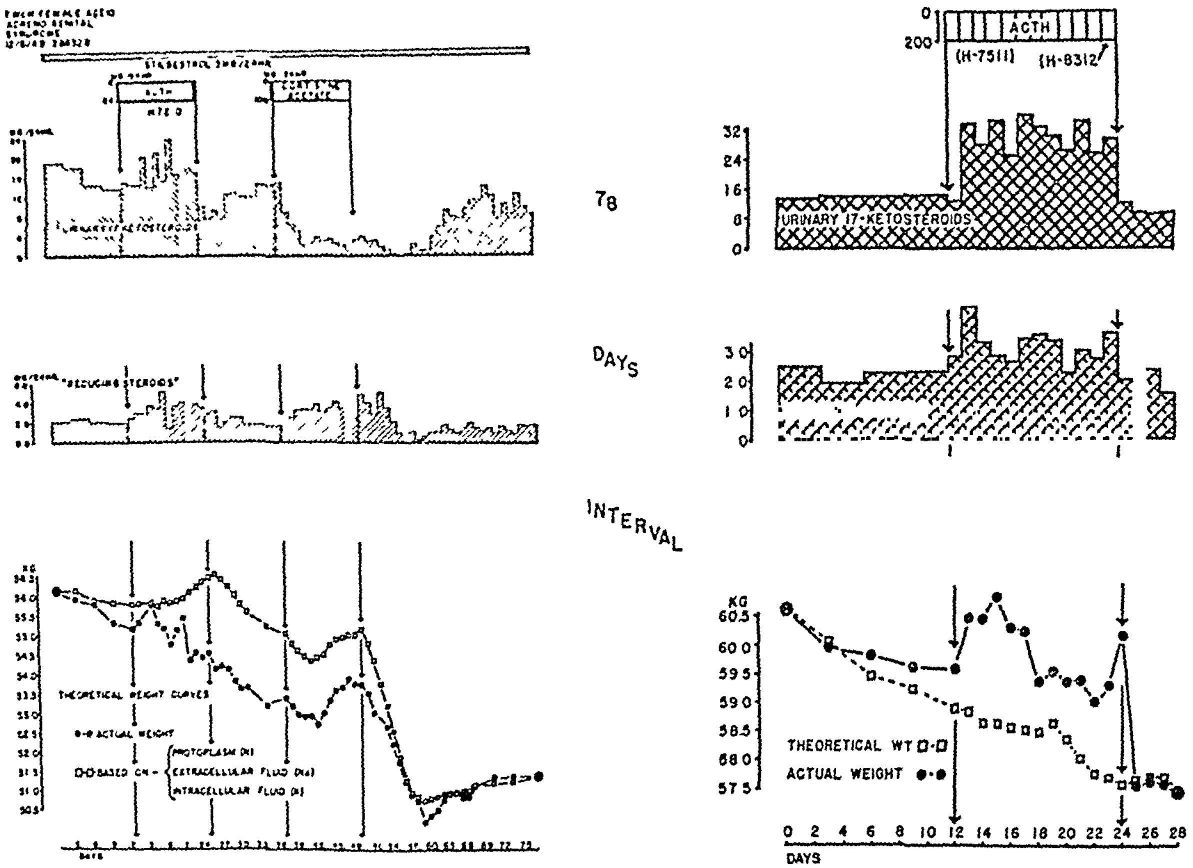

FIG. 6. URINARY 17-KETOSTEROIDS AND "REDUCING STEROIDS," ACTUAL AND THEORETICAL WEIGHT BASED ON N, NA, AND K DURING EXPERIMENT SHOWN IN FIGURE 4

The upper limit of normal for reducing steroids by this method is 5 mg. per 24 hours.

sodium-retaining properties of the adrenal steroids and the sodium diuretic properties of contaminating posterior pituitary substances (7, 17). Similar doses of these preparations of ACTH produce sodium retention in patients with essentially normal adrenal cortical function, in spite of the presence of pitressin in quantities equal to or greater than those given here. Thus there is no evidence in these patients that sodium-retaining steroids were released in response to ACTH.

Lewis and Wilkins (18) reported sodium loss following administration of ACTH for a single day to two patients with the adrenogenital syndrome. The amount of contaminating pitressin their patients received was relatively high. It should be noted, however, that sodium retention very frequently fails to occur during the first 24 hours of ACTH therapy to patients who later show marked retention (4–7).

*f) Magnesium metabolism*

Mg balances were measured during three of the four studies with ACTH. When a daily dose of 200 mg. was given (Figure 5) urinary Mg fell. In the other experiments (Figures 5 and 8) no consistent effect on Mg metabolism was noted.

*g) 17-Ketosteroids*

In all subjects the 17-ketosteroid excretion was very high (19) for the age of the patient. In patient C. O., ACTH produced no further rise in 17-ketosteroid excretion (Figure 3); in T. McH., a questionable rise was produced with a dosage of 84 mg., and a marked rise with 200 mg. a day (Figure 6). In patient R. H. a marked rise was produced with 84 mg. a day (Figure 9).

*h) Reducing steroids ("11 oxysteroids")*

ACTH did not produce a significant rise in reducing steroids in any of the experiments (Figures

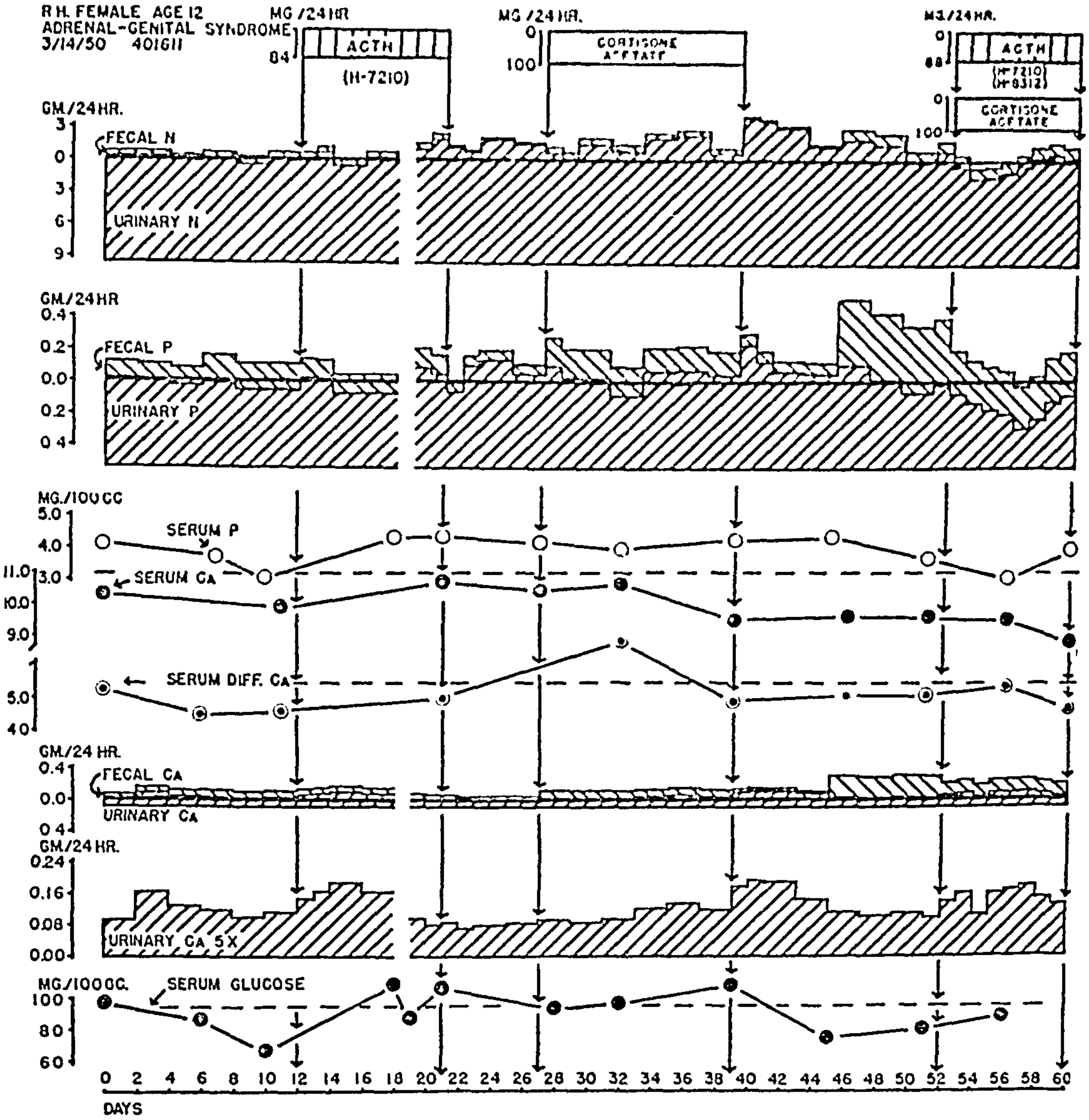

Fig. 7. N, P, and Ca Balances, Urinary Ca and Serum P, Ca, and Diffusible Ca and Glucose During Administration of ACTH and Cortisone to R. H., a 12 Year Old Female with the Adrenogenital Syndrome

3, 6, and 9). This result is in striking contrast to the elevations of urinary reducing steroids of two to ten times the normal values which have been consistently reported following similar doses of ACTH (3, 4, 6, 15).

### *i) Circulating eosinophils*

The initial counts of fasting eosinophils were high in the two patients (T. McH. and R. H.) in whom they were done. With ACTH (Figure 12) there was a transient fall, followed by a rise to or above the initial values, despite continued ACTH therapy. This observation is in contrast to the well-documented observation (3–6) that the circulating eosinophils fall to approximately zero and remain there with comparable doses of ACTH in patients with essentially normal adreno-cortical function.

### *j) Glycosuria*

No patient developed glycosuria at any time during therapy.

### *Summary of results of ACTH therapy*

In many respects, these patients responded abnormally to ACTH. The failure to increase significantly the excretion of reducing steroids, to develop a negative N balance, or to show a large and sustained fall of circulating eosinophils is an indication that the output of "carbohydrate-active" steroids[6] ("sugar" hormone) following ACTH is markedly subnormal. The failure to develop transient K diuresis is an indication that the output of either "carbohydrate-active" or "salt-retaining" steroids or both is subnormal—if, indeed, these are separate hormones (20). The failure to develop Na and Cl retention is an indication that the output of "salt-retaining" steroids is similarly impaired.

In one respect these patients responded normally to ACTH. In spite of high initial levels, the urinary 17-ketosteroid excretion rose in all experiments except one, that on C. O. In this experiment the initial level was highest, and the lowest dose of ACTH was given. The urinary 17-ketosteroids are presumably (2) an index of the adrenal androgen which is responsible for the virilism in these patients, and the initially high values are of course a cardinal feature of the disease.

These findings suggested two metabolic disorders in the pathogenesis of this condition: diminished ability of the adrenal cortex to respond to ACTH with an increased output of carbohydrate-active and salt-retaining steroids, and an increased production of ACTH (*vide infra*) resulting in the abnormally high production of adrenal androgen (see also Figure 13).

It was then postulated that a carbohydrate-active adrenal cortical hormone such as cortisone should depress the production of ACTH and thus diminish adrenal androgen production. Conse-

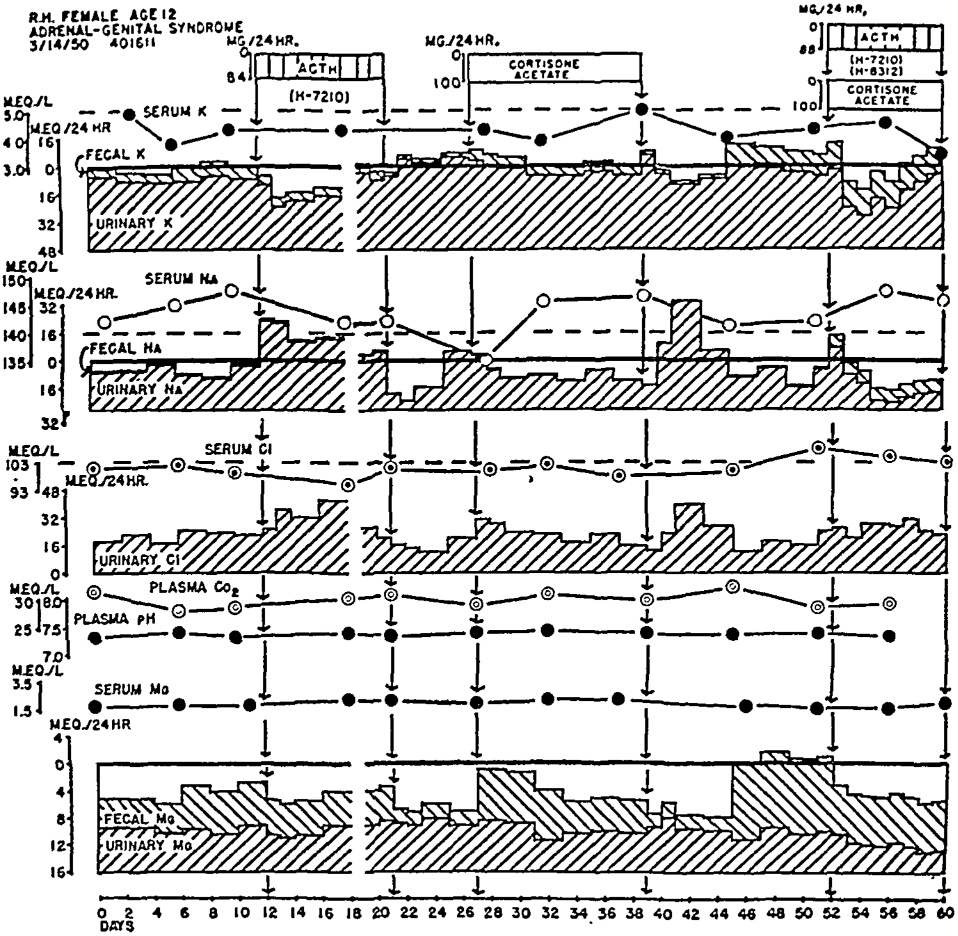

FIG. 8. K, NA, AND MG BALANCES, URINARY CL, SERUM K, NA, CL, $CO_2$ CONTENT, AND MG, AND PLASMA PH DURING THE EXPERIMENT SHOWN IN FIGURE 7

quently, cortisone acetate was administered in doses of 100 mg. a day for 12 days to patients T. McH. and R. H.

---

[6] Probably these consist of Compound F, Compound E, or closely related steroids.

### *II. Cortisone*

The metabolic effects of cortisone are shown in Figures 4–9. The changes in P, Ca, K, Na, and Cl metabolism and in excretion of reducing steroids are not remarkable, and do not differ essentially from those previously reported (6) following the use of cortisone acetate at similar dosages. There was a slight fall of urinary Mg in both patients.

Cortisone produced a rise in urinary N in both patients, resulting in a markedly negative N balance in T. McH. (Figure 4) and increasing the already existing negative N balance in R. H. (Figure 7). This result is contrary to those of Sprague and associates (6), Clark, Ropes and Bauer (21), and ourselves (22), following the use of cortisone at this dosage in patients with other syndromes. Sprague and his co-workers state, "As a rule, the augmentation of nitrogen excretion was slight or absent when the dose of cortisone was 100 mg. daily."

The second striking result of cortisone therapy was the marked progressive fall in 17-ketosteroid excretion in both patients (Figures 6 and 9). The urinary 17-ketosteroids remained low for eight (R. H.) and 12 (T. McH.) days, then rose gradually to the pre-injection levels. A similar depression of 17-ketosteroids with cortisone in a patient with the adrenogenital syndrome has been reported by Wilkins and his colleagues (23).

### *III. ACTH and Cortisone*

As stated above, we had believed, *a priori*, that the effect of cortisone would be to lower the uri-

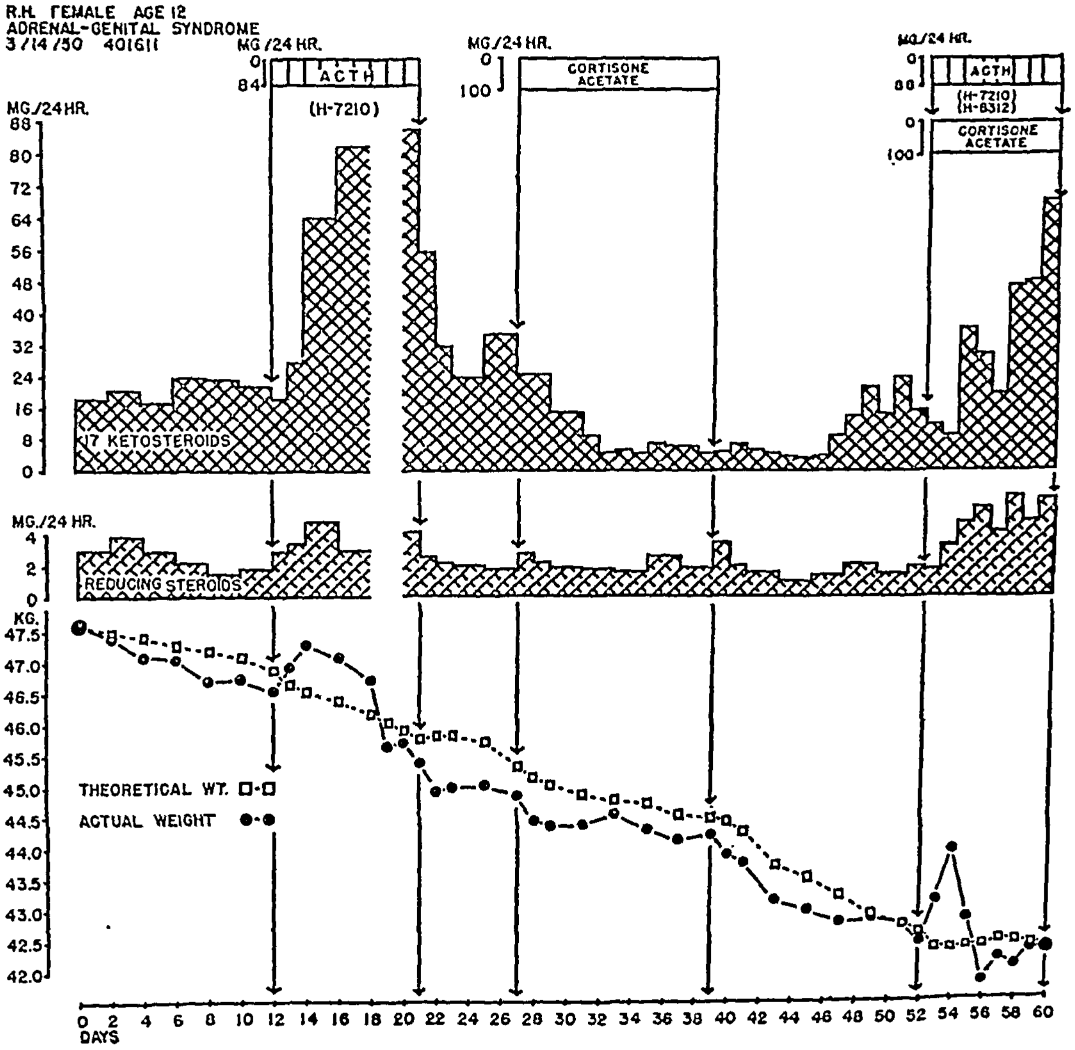

FIG. 9. URINARY 17-KETOSTEROIDS, "REDUCING STEROIDS," ACTUAL WEIGHT, AND THEORETICAL WEIGHT BASED ON N, NA, AND K DURING EXPERIMENT SHOWN IN FIGURE 7

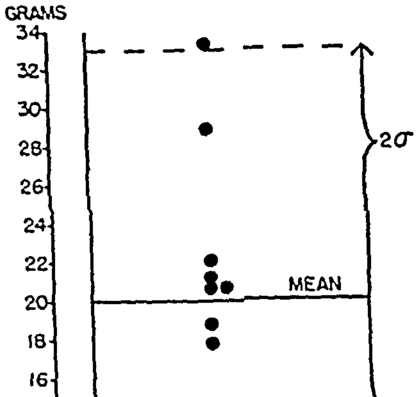

FIG. 10. THE TOTAL N EXCRETION DURING THE FIRST SIX DAYS OF ACTH ADMINISTRATION TO THE THREE PATIENTS REPORTED, CONTRASTED WITH THAT IN 11 COMPARABLE EXPERIMENTS ON PATIENTS WITH ESSENTIALLY NORMAL ADRENAL CORTICAL FUNCTION, AND ONE ON A PATIENT WITH MYXEDEMA

Nitrogen excretions were calculated as deviations from the average of control values. Note decreased N excretion in patients with the adrenogenital syndrome, and actual N retention with 200 mg. of ACTH in T. McH. (2).

nary 17-ketosteroid excretion by depressing pituitary ACTH production, and not by direct action on the adrenal gland. To provide further evidence on this point we decided to administer ACTH and cortisone together to patient R. H. If cortisone has a direct action on the adrenal cortex it should decrease the response to administered ACTH. The results show that the rise in 17-ketosteroid excretion resulting from ACTH together with cortisone (Figure 9, days 53 through 60) did not differ essentially from that resulting from ACTH alone (Figure 9, days 13 through 20). Thus they do not show direct action of cortisone on the adrenal cortex or on its response to ACTH in this patient. Hence, they support the view that cortisone acts by inhibiting endogenous ACTH.

## DISCUSSION

### a) *Nitrogen metabolism*

Consideration of the adrenogenital syndrome, which may be associated with either hyperplasia or tumor of the adrenal cortex, leaves little doubt that the adrenal cortex may produce steroids which have virilizing properties. There is considerable circumstantial evidence (2) that the measurement of urinary 17-ketosteroids provides an index of the quantities of such steroids being produced. Further, there is clinical evidence (2) that these steroids may have somatotropic, nitrogen-retaining, as well as virilizing properties.

The present study provides two pieces of indirect evidence in support of the latter point. In the first place, when T. McH. received 200 mg. of ACTH a day, there was a marked rise in 17-ketosteroid excretion without a significant rise in urinary reducing steroids. Under these circumstances, nitrogen retention was increased.[7] Nitrogen retention with ACTH has not heretofore been described, presumably because nitrogen loss with "sugar" hormone outweighs any nitrogen retention due to somatotropic hormones. In the second place, when T. McH. and R. H. received 100 mg. of cortisone a day there was a marked fall in 17-ketosteroid excretion. Under these circumstances, more loss of N was produced than has been observed following this dosage[8] of cortisone. Thus in the first instance, with a rise of adrenal androgen unopposed by adrenal "sugar" hormone there was N retention, whereas in the second instance, with a fall of adrenal androgen there was an abnormally great N loss following exogenous "sugar" hormone.

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[7] N retention did not occur in R. H. in spite of a greater rise in 17-ketosteroid excretion. This may be a result of qualitative differences in the precursors of the 17-ketosteroids in these two patients (13, 24).

[8] Either on an absolute or a mg. per kg. basis.

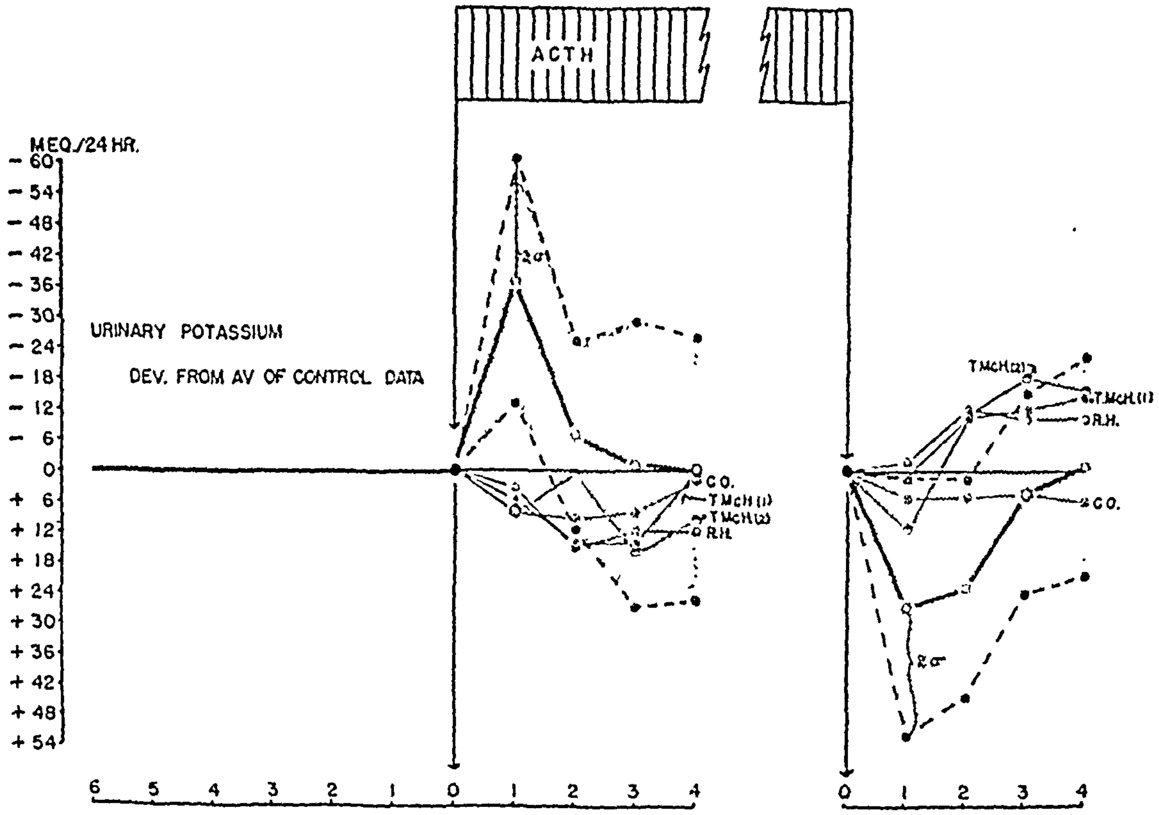

FIG. 11. THE URINARY K EXCRETION DURING THE FIRST FOUR DAYS OF, AND THE FIRST FOUR DAYS AFTER, ADMINISTRATION OF ACTH TO THE PATIENTS REPORTED CONTRASTED WITH THAT IN 11 COMPARABLE EXPERIMENTS ON PATIENTS WITH ESSENTIALLY NORMAL ADRENAL CORTICAL FUNCTION

The shaded area is that covered by two standard deviations above and below the mean normal curve (*heavy black line*). Note that patients with the adrenogenital syndrome did not show the K diuresis on starting nor the K retention on stopping ACTH.

### *b) Clinical implications*

During these short metabolic studies, no clinically apparent change in these patients was expected or found. Subsequent to these studies, T. McH. has received continuously 100 mg. of cortisone twice a week (total duration, five months to date of writing) and R. H. 50 mg. twice a week (total duration, six months to date of writing).

In neither patient has this therapy produced a fall in the number of circulating eosinophils; in both, it has maintained the 17-ketosteroid excretion at a level approximately normal (19) for the patient's age and sex. T. McH. has begun to have regular periods with cramps. It is too early to say whether further virilism can be prevented by this form of therapy.

### *c) Interpretation of the pathological physiology*

There are a number of possible explanations for the findings reported here. One such explanation is shown graphically in Figure 13. It is postulated that adrenal androgen inhibits pituitary ACTH production relatively weakly, whereas "sugar" hormones inhibit ACTH relatively strongly. It is suggested that the primary defect in this syndrome is the decreased production of "sugar" hormone by the adrenal cortex in response to ACTH. One then has the following sequence of events:

*1*) lack of "sugar" hormone,
*2*) failure of normal inhibition of ACTH,
*3*) increased output of ACTH, and
*4*) resulting increase of adrenal androgen.

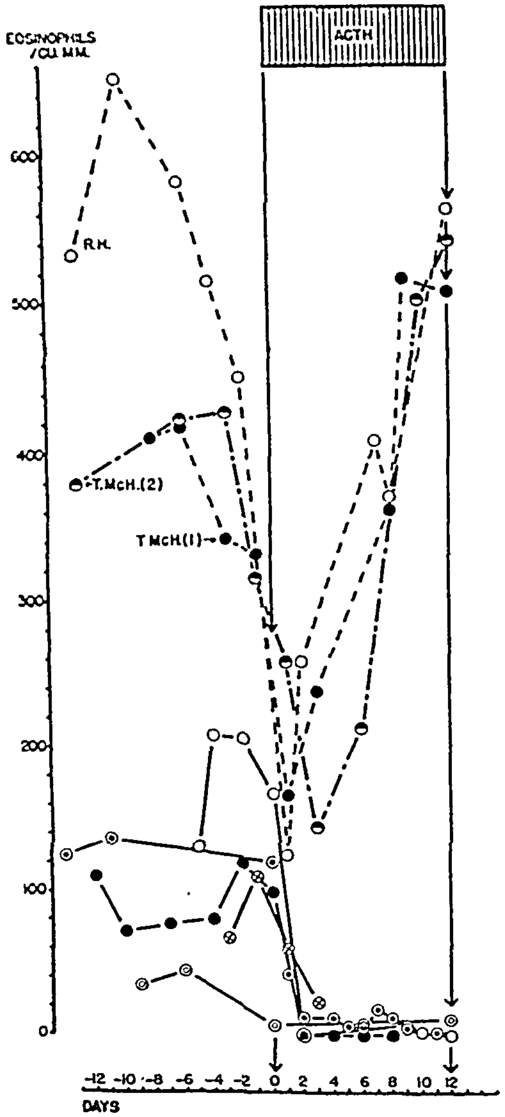

FIG. 12. THE EFFECT OF ACTH ON THE CIRCULATING EOSINOPHILS IN THREE OF THE EXPERIMENTS REPORTED (BROKEN LINES) CONTRASTED WITH THAT IN FIVE COMPARABLE EXPERIMENTS ON PATIENTS WITH ESSENTIALLY NORMAL ADRENAL CORTICAL FUNCTION (SOLID LINES)

Note the high initial level, and the comparatively slight and transitory fall in the patients with the adrenogenital syndrome.

This schema does not require that the patients show an absolute deficit of "sugar" hormone, since the increased production of ACTH may completely compensate for the inherent insensitivity to ACTH.

That there is an increased production of ACTH in this syndrome has not been established. Dr. A. Albert (27) tested the serum of T. McH. and could not demonstrate the presence of ACTH. He did, however, demonstrate the presence of increased amounts of ACTH in the serum of one adult patient from this clinic with a classical case of the adrenogenital syndrome.

## SUMMARY AND CONCLUSIONS

1. Four courses of ACTH were administered to three female patients with the adrenogenital syndrome resulting from congenital adrenal hyperplasia. The dosage ranged from .71 to 3.33 mg. per day per kg. body weight, the duration from nine to 12 days. Studies included urinary 17-ketosteroids, reducing steroids, glucose, and chloride, balances of N, P, Ca, K, Mg, and Na, and measurements of circulating eosinophils, serum P, Ca, K, Mg, Cl, $CO_2$ and glucose.

2. ACTH produced a rise in urinary 17-ketosteroids in three of the four courses, but none of *its other usual metabolic effects. With the highest* dosage of ACTH there was actual nitrogen retention.

3. Cortisone acetate was administered to two of these patients on the same regimen. The dosages were 1.90 and 2.22 mg. per day per kg. body weight, the duration 12 days.

4. Cortisone produced a fall to normal and even below normal in the elevated urinary 17-ketosteroid excretion, and a negative N balance. The latter is exceptional at the dosages employed.

5. ACTH (2.05 mg. per kg. per day) and cortisone (2.32 mg. per kg. per day) were administered together to one of these patients for eight days. The resulting rise in urinary 17-ketosteroids was essentially the same as that produced by ACTH alone.

6. Cortisone acetate is being administered continuously to two of these patients at dosages of 100 and 200 mg. a week. At this dosage the urinary 17-ketosteroids remain normal and the circulating

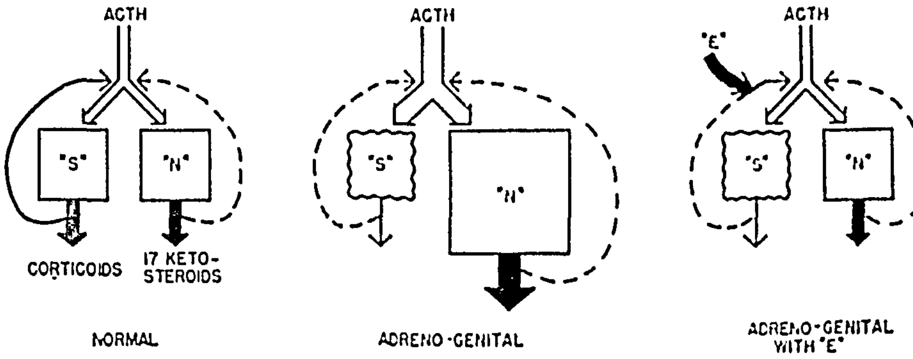

FIG. 13. SCHEMA ILLUSTRATING A POSSIBLE EXPLANATION FOR THE PATHOLOGICAL PHYSIOLOGY OF THE ADRENOGENITAL SYNDROME AND THE EFFECT THEREON OF COMPOUND E

The square figures represent the adrenal cortex. "S" refers to carbohydrate-active ("sugar") hormones, precursors of urinary corticoids; "N" refers to "adrenal androgens," precursors of urinary 17-ketosteroids.

It is postulated that "S" hormones inhibit ACTH effectively (solid arrows directed backwards toward ACTH), whereas "N" hormones do not (broken arrows directed backwards toward ACTH). For further discussion see text.

eosinophils are not depressed. One patient is having monthly periods with cramps.

7. The above findings suggest that this syndrome involves a primary defect of the adrenal cortex, and a secondary increase of pituitary ACTH production.

8. In all patients the electroencephalograms showed asymmetry, elevation of voltages, and abnormal slow waves. This finding is unexplained.

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## ERRATUM

In Table VII of the article entitled "Acetylation Studies in Human Subjects with Metabolic Disorders," by H. Gershberg and W. J. Kuhl, Jr., in the December 1950 issue (pp. 1625–1632), the values for both total and free serum cholesterol are *mgm. per cent* rather than as erroneously given.

# THE LABILE FACTOR OF PROTHROMBIN CONVERSION: ITS CONSUMPTION UNDER NORMAL AND PATHOLOGICAL CONDITIONS AFFECTING BLOOD COAGULATION [1, 2]

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(Submitted for publication September 28, 1950; accepted, December 11, 1950)

## INTRODUCTION

The rapid conversion of prothrombin to thrombin by thromboplastin plus calcium requires a plasma component called "Labile Factor" (2, 3), which is probably identical with plasma Ac-globulin and Factor V (4, 5), and with the prothrombin accelerator of Fantl and Nance (6, 7).[3] Deterioration of this substance as plasma ages produces an increase in the prothrombin time (2, 3, 8) although the concentration of prothrombin remains essentially unchanged (9, 10). This defect can be rectified by the addition of fresh plasma devoid of prothrombin as well as of fibrinogen (11, 8).

Additional information regarding Labile Factor (L.F.) is desirable for several reasons. Its precise role in prothrombin conversion is obscure, and hemorrhage referable to deficiency of this entity or of a similar substance has been described (12–14). Furthermore, as will become evident, the concentration of L.F. must receive due consideration in procedures which measure prothrombin.

In certain hemorrhagic diseases the conversion of prothrombin to thrombin is reduced or retarded (15, 16). The "prothrombin consumption test," which demonstrates this defect, promises to become important in the investigation and diagnosis of such disturbances. In order to compute the consumption of prothrombin during coagulation, the precise amount of prothrombin in both serum and its parent plasma must be known. Obviously changes in the concentration of L.F. in the transition from plasma to serum may influence considerably the velocity of prothrombin conversion in serum and, therefore, the determination of serum prothrombin by the one-stage procedure, since the prothrombin times may reflect the concentration of L.F. as well as that of prothrombin itself. Under such circumstances the computation of prothrombin consumption from differences between plasma and serum prothrombic activities may be erroneous.

Whereas the concentration of L.F. has been determined in the *plasma* of man and other species (17), little is known regarding its level in *serum*. This information is presented in the present report. Also included is a method proposed for the determination of serum L.F. The data indicate that during and after coagulation changes in this clotting factor occur which are considerably influenced by conditions, pathological or induced, which affect clotting.

## METHODS

*Prothrombin determination:* The prothrombic activity of oxalated plasma (one volume of 0.1 M sodium oxalate to nine volumes of blood) was determined on undiluted plasma by the orthodox one-stage procedure (18), and on diluted plasma by the modification (19) in which the test plasma is diluted with fresh normal oxalated pooled plasma rendered prothrombin-free by adsorption with $BaSO_4$.[4] Prothrombin levels were calculated by interpolating the observed prothrombin times on standardization curves obtained on normal plasma similarly diluted. Serum prothrombin was measured similarly and also

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[1] Supported by a grant from The Commonwealth Fund.

[2] Presented in part before the Federation of American Societies for Experimental Biology, April, 1950 (1).

[3] It should be pointed out that most investigators feel that Labile Factor, Factor V, plasma Ac-globulin, and the factor of Fantl and Nance are identical. Thus Quick and Stefanini (31) use the terms interchangeably to designate one entity. Seegers and his associates (10) are inclined to agree that Ac-globulin is the same as L.F. and Factor V (30, 40). We (24) as well as Flynn and his coworkers (41) hold the same view. Definite proof regarding the identity of all these moieties, however, is still lacking.

[4] Plasma so treated is hereafter referred to as "$BaSO_4$ plasma."

by the modified two-stage method of Ware and Seegers (20).

*Labile factor:* Pooled oxalated plasma from normal subjects was stored at 4-5°C for variable intervals during which its prothrombin time increased progressively. L.F. activities of test plasma or sera were measured by their restorative effects on the elevated prothrombin time of these aged plasmas in which the factor had largely deteriorated (8, 17). The restorative effect was determined by adding up to one part of plasma or serum (whole or diluted with physiological saline) to four parts of the aged plasma, and by observing the prothrombin times of the mixtures. The values herein reported are the averages of several observations on each mixture. In ranges below 45 seconds the prothrombin times were always duplicable within two seconds; between 45 and 60 seconds, within four seconds.

Except where otherwise indicated, the serum was separated one hour after venous blood was allowed to clot in a glass tube at room temperature. The clot was rimmed and centrifuged, and the serum was removed and incubated at 37°C for 15 minutes in order to inactivate thrombin. The time interval between incubation of the serum and determination of its activity was noted. Experiments were run on non-oxalated as well as oxalated serum (1 oxalate to 4 serum). In some instances citrate (2.5 per cent sodium citrate) was used instead of oxalate. Also studied were sera which were subjected to adsorption with $BaSO_4$ for 10 minutes at 37°C. Controls for the latter experiments were incubated for the same interval without $BaSO_4$.

Commercial thromboplastin (Difco) and beef lung thromboplastin were used in the one- and two-stage methods, respectively.

## RESULTS

The experiments reported below are typical of a series of experiments performed under the same conditions.

### *Comparative effect of normal serum and plasma on prothrombic activity of aged plasma*

Oxalated fresh plasma has a profound restorative effect on the retarded prothrombin conversion of stored plasma (Figure 1). This is not due to the prothrombin in the fresh plasma, since removal of the prothrombin by $BaSO_4$ adsorption does not significantly decrease its restorative activity (Figure 1, Table I). There appears to be remarkably little variation in the L.F. activity of plasma from different normal subjects (Table I). These observations confirm earlier reports (8, 11, 21).

Non-oxalated normal serum tested 75 minutes after coagulation, has as much, and often more, restorative activity than the parent plasma tested simultaneously (Table II). On the other hand,

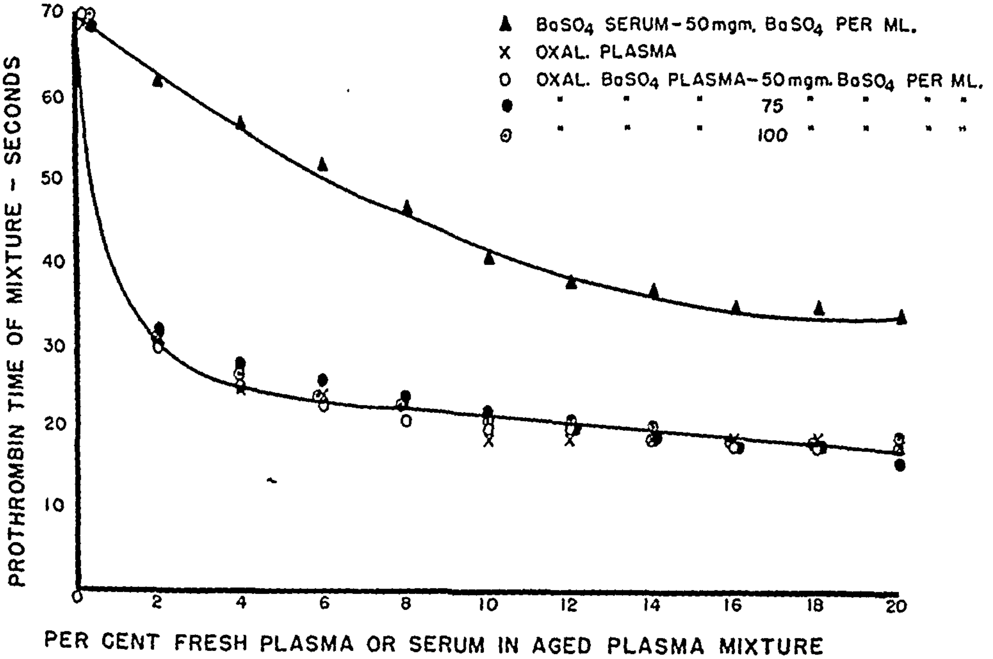

FIG. 1. RESTORATIVE ACTIVITY OF FRESH NORMAL PLASMA, $BaSO_4$ NORMAL PLASMA, AND $BaSO_4$ NORMAL SERUM ON RETARDED PROTHROMBIN CONVERSION OF AGED PLASMA (SIX WEEKS AT 4°C)

TABLE I

*Variation of L.F. in fresh normal oxalated plasma before and after adsorption with $BaSO_4$*

| Subject..... | Prothrombin time (*sec.*) of plasma mixture | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | | 2 | | 3 | | 4 | |
| Per cent fresh plasma in fresh plasma-aged plasma mixture | Whole plasma | $BaSO_4$ plasma | Whole plasma | $BaSO_4$ plasma | Whole plasma | $BaSO_4$ plasma | Whole plasma | $BaSO_4$ plasma |
| 0 | 87 | 87 | 87 | 87 | 87 | 87 | 87 | 87 |
| 1 | 43 | 46 | 42 | 46 | 41 | 42 | 40 | 44 |
| 2 | 36 | 37 | 35 | 36 | 34 | 37 | 35 | 35 |
| 4 | 29 | 29 | 28 | 30 | 27 | 30 | 27 | 30 |
| 6 | 27 | 28 | 24 | 27 | 25 | 29 | 26 | 28 |
| 10 | 23 | 26 | 23 | 24 | 21 | 25 | 21 | 24 |
| 20 | 19 | 21 | 20 | 22 | 20 | 21 | 19 | 21 |

$BaSO_4$ serum is considerably less potent than the control serum (Table III). From interpolation of these data on the curve in Figure 1, it is evident that $BaSO_4$ removes approximately 80 per cent or more of the restorative activity from serum whereas it does not do so from plasma.

Serum has certain clot-promoting properties not demonstrable in plasma (22). This is due to the serum prothrombin conversion accelerator (spca) which arises during coagulation and which in the presence of L.F. accelerates prothrombin conversion (22–24). Accordingly, the accelerating effect of serum on the prothrombin conversion in aged plasma might be due to both spca and L.F.

$BaSO_4$ removes little, if any, L. F. from plasma (8, 21). It is assumed that it likewise does not remove this substance from serum. On the other hand, spca is quantitatively adsorbed (22, 24). Accordingly, $BaSO_4$ serum is devoid of spca but should retain its full complement of L.F. That $BaSO_4$ serum has less restorative ability than $BaSO_4$ plasma indicates, therefore, that the L.F. of normal serum is far less than that of plasma.

### *Effect of anti-coagulants upon restorative property of normal serum*

L.F. is more stable in citrated than in oxalated plasma (8, 25). It is also more stable in native plasma (containing calcium) but not in plasma rendered incoagulable by decalcifying with ion-exchange resins (26). Experiments were performed in order to see whether the L.F. in serum is similarly affected (Table IV). The data indicate that the restorative property of oxalated serum is more labile than that of non-oxalated serum.

The question arises whether oxalate affects L.F., not adsorbable by $BaSO_4$, or some other serum factor such as spca. Experiments performed on non-oxalated versus oxalated $BaSO_4$ serum

TABLE II

*Restorative activity of normal plasma and corresponding sera* on retarded prothrombin conversion of aged plasma*

| Proth-T† (*sec.*) of mixture‡ | | |
|---|---|---|
| Sal. | Fresh oxal. plas. | Ser. |
| 113 | 18 | 19 |
| 66 | 18 | 17 |
| 33 | 21 | 17 |
| 66 | 17 | 18 |
| 37 | 22 | 16 |
| 45 | 18 | 16 |

* All sera separated one hour after clotting at room temperature in glass; both sera and plasma incubated at 37°C for additional 15 minutes and then tested.

† Prothrombin time.

‡ Mixture comprises 0.4 ml. of aged plasma plus 0.1 ml. of material indicated.

TABLE III

*Effect of $BaSO_4$ adsorption on restorative activity of normal sera (S)* on prothrombin conversion of aged plasma (A.P.)*

| Interval between clotting and test | Proth-T (*sec.*) of mixture | | |
|---|---|---|---|
| | A.P. | A.P. plus S | A.P. plus $BaSO_4$ S† |
| *hrs.* | | | |
| 3½ | 33 | 19 | 31 |
| 2 | 45 | 21 | 31 |
| 3½ | 37 | 21 | 34 |
| 2 | 48 | 21 | 32 |
| 1½ | 66 | 22 | 42 |

* Non-oxalated sera, separated one hour after blood was shed. One part serum added to four parts A.P.

† Sera adsorbed with 50 mgm. $BaSO_4$ per ml.

TABLE IV

*Deterioration of serum L.F. in presence of oxalate* and citrate**

Prothrombin time (sec.)

| | 75 min. after coag. | | | 195 min. after coag. | | |
|---|---|---|---|---|---|---|
| | I | II | III | I | II | III |
| Aged plas. | 37 | 37 | 37 | 37 | 37 | 37 |
| Aged plas. plus:† | | | | | | |
| Ser. | 16 | 15 | 14 | 21 | 17 | 18 |
| Cit. ser. | 17 | 18 | 18 | 21 | 24 | 22 |
| Oxal. ser. | 18 | 19 | 19 | 28 | 40 | 38 |

* Oxalate and citrate added to normal serum 60 min. after coagulation.

† 0.1 ml. serum added to 0.4 ml. of aged plasma.

(Table V) indicate that the latter, also, was more labile than its non-oxalated counterpart. On the basis of the assumption that $BaSO_4$ does not remove L.F. from serum, it is concluded that oxalate accelerates deterioration of this clotting component.

The restorative activity seems more stable in citrate than in oxalate but not as stable as in native serum (Table IV). It appears, therefore, that L.F. deteriorates more rapidly in serum containing oxalate than in native or citrated serum. The same is observed in plasma (8, 17, 25, 26).

TABLE V

*Effect of oxalate on L.F. activity of normal serum (S)*

| Aged plasma plus:* | Proth-T (*sec.*) |
|---|---|
| Saline | 61 |
| Fresh S | 17 |
| Fresh $BaSO_4$ S | 31 |
| Fresh oxal. S | 19 |
| Fresh oxal. $BaSO_4$ S | 38 |
| S kept 3 hrs., room temp. | 20 |
| $BaSO_4$ S kept 3 hrs., room temp. | 42 |
| Oxal. S kept 3 hrs., room temp. | 32 |
| Oxal. $BaSO_4$ S kept 3 hrs., room temp. | 55 |

Sera separated one hour after clotting.
$BaSO_4$ sera prepared with 100 mgm. $BaSO_4$ per ml.

* One part material added to four aged plasma (60 days at 4–5°C).

### *Consumption of L.F. and prothrombin during normal coagulation*

That L.F. disappears from blood during coagulation is clearly established. Its rate of disappearance was studied in relation to the rate of prothrombin consumption. Aliquots of freshly drawn blood were allowed to clot at 37°C in separate dry test tubes. At specified intervals oxalate was added, the mixture was stirred with a glass rod and immediately centrifuged at 2000 r.p.m. for 10 minutes. The sera were separated and subjected to determination of residual prothrombic activity

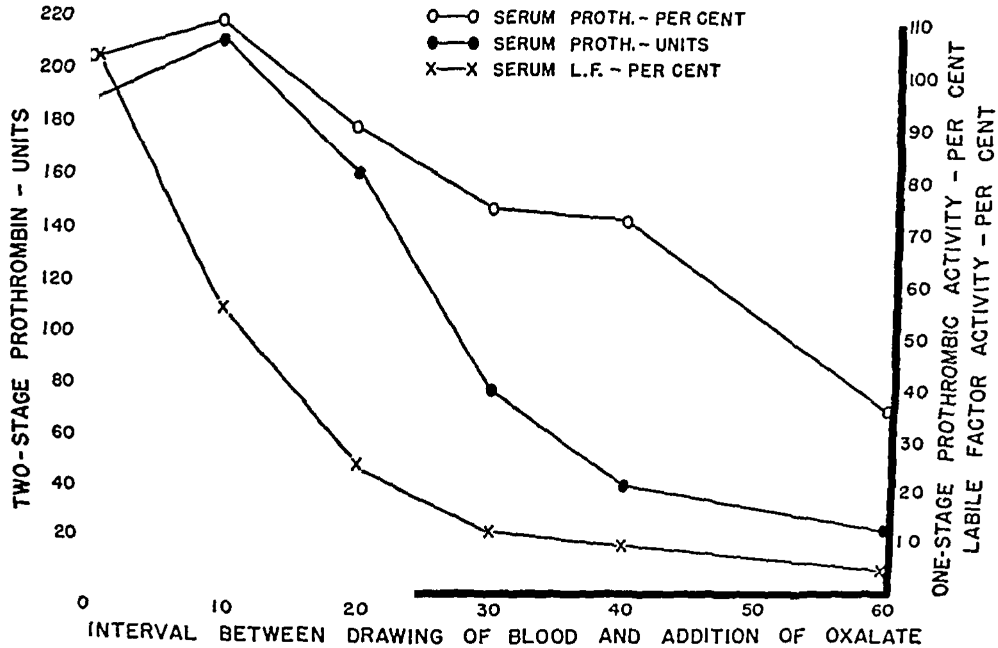

Fig. 2. Labile Factor, and One-Stage and Two-Stage Prothrombic Activities of "Serum" during Coagulation of Normal Blood

Aged plasma on which L. F. activities were tested had a prothrombin time of 65 sec.

TABLE VI

*Effect of thromboplastin (TPL) supplement to clotting blood on L.F. in normal serum (S) (non-oxalated)*

| Aged plasma plus: | Proth-T (*sec.*) Exp't 1* | Proth-T (*sec.*) Exp't 2† |
|---|---|---|
| Saline | 47 | 63 |
| S | 25 | 33 |
| $BaSO_4$ S | 30 | 44 |
| TPL S | 34 | 47 |
| TPL $BaSO_4$ S | 39 | 64 |

$BaSO_4$ S prepared with 25 mgm. $BaSO_4$ per ml. TPL S obtained from blood (nine vol.) added to TPL solution (one vol.) prepared as for prothrombin determination. Identical results were obtained when the ratio was 19 to 1.

* One volume of material to four of aged plasma.
† One volume of material to one of aged plasma.

by both one- and two-stage methods. Aliquots of the serum were also adsorbed with $BaSO_4$ (50 mgm. per ml.) and were then tested for L.F. activity. It is evident (Figure 2) that both L.F. and prothrombin disappear during the coagulation process. Whereas L.F. began to decrease promptly after the blood was shed, the two-stage prothrombin values showed no significant change within the first 10 minutes. Within 40 minutes most of both clotting factors had disappeared. There was considerable discrepancy between the one- and two-stage prothrombin values. This is not surprising since one-stage prothrombin times of serum reflect the concentration not only of unconsumed prothrombin but also of residual L.F. together with evolved spca.

It is of interest that within the first 10 minutes, about one-half of the L.F. had disappeared, before any detectable (by the methods used) decrease in prothrombin. Although this must be significant in the role of L.F. in prothrombin conversion, the evidence is too meager to permit any interpretation.

### *Effect of thromboplastin on concentration of L.F. in serum*

Since thromboplastin added to normal blood yields serum which is always devoid of prothrombin in contrast to normal sera which often show small amounts, it was of interest to study the L.F. content of such sera. Sera from thromboplastin-supplemented blood were far less able to restore prothrombic activity to aged plasma than sera from spontaneously clotted blood (Table VI). This

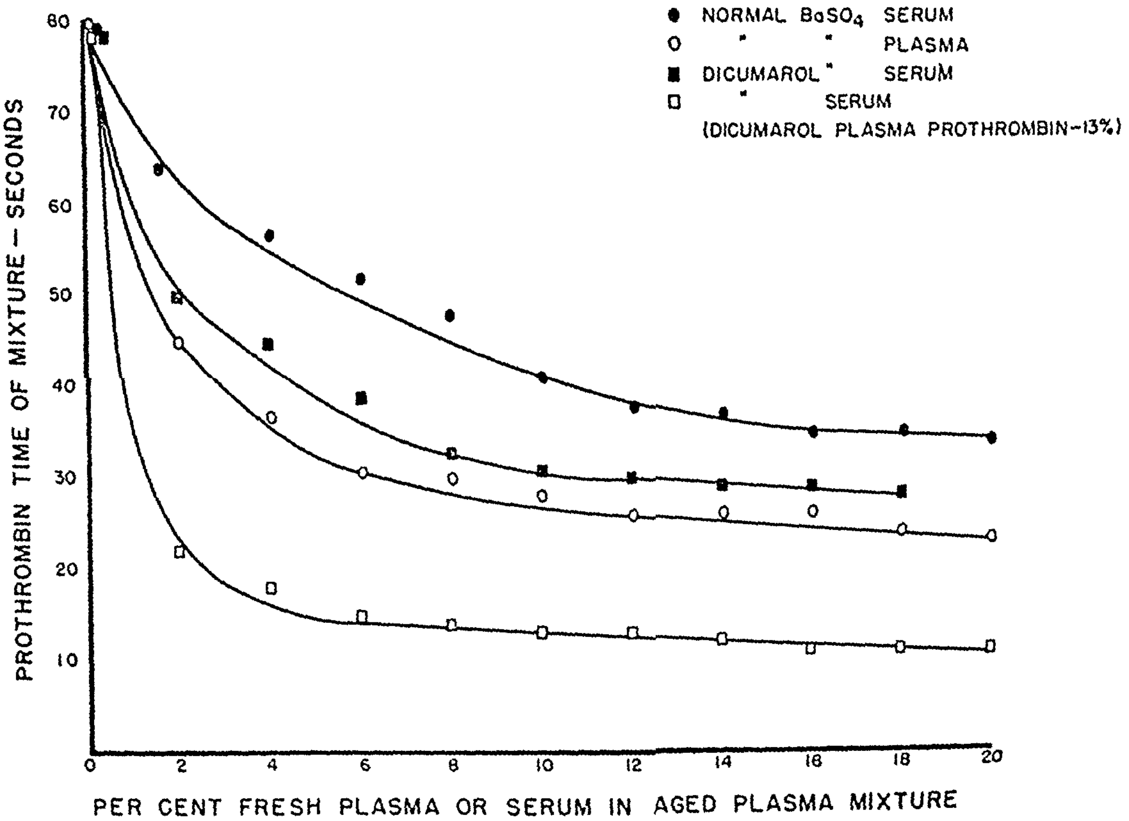

FIG. 3. RESTORATIVE ACTIVITY OF "DICUMAROL" SERUM ON RETARDED PROTHROMBIN CONVERSION OF AGED NORMAL PLASMA

Serum was non-oxalated, and was tested 1½ hours after the blood was obtained. Adsorption with $BaSO_4$ was carried out with 50 mgm. per ml.

difference, evident also in the $BaSO_4$ adsorbed sera, suggests that more L.F. is consumed when coagulation and prothrombin conversion are accelerated by thromboplastin.

### *L.F. in serum from dicumarolized blood*[5]

The purpose of these experiments was to study L.F. depletion during coagulation in relation to the amount of prothrombin available for conversion to thrombin. Dicumarolized blood contains essentially normal amounts of L.F. (17). As is evident in Figure 3, dicumarol serum has a very strong restorative effect on aged plasma, more potent than normal serum (compare with data of Table II; also compare data in Table VII with those in Table V, all obtained on the same aged plasma). The same is true of dicumarol $BaSO_4$ serum compared with its normal counterpart. Again, as with normal serum, oxalate accelerated L.F. deterioration (Table VII).

TABLE VII

*L.F. activity of dicumarol serum (D.S.) with and without oxalate*

| Aged plasma* plus: | Proth-T (*sec.*) |
|---|---|
| Saline | 61 |
| Fresh D.S. | 11 |
| Fresh oxal. D.S. | 14 |
| Fresh $BaSO_4$ D.S. | 26 |
| Fresh $BaSO_4$ D.S.-oxal. | 39 |
| D.S. kept 3 hrs., room temp. | 13 |
| Oxal. D.S., 3 hrs., room temp. | 22 |
| $BaSO_4$ D.S., 3 hrs., room temp. | 36 |
| $BaSO_4$ oxal. D.S., 3 hrs., room temp. | 64 |

The prothrombic activity of the parent plasma was 11 per cent. Serum separated one hour after clotting. $BaSO_4$ S prepared with 100 mgm. $BaSO_4$ per ml.

* One volume of material to four of aged plasma (60 days at 4–5°C).

It was of interest to study the stability of L.F. in dicumarol serum as contrasted with normal serum. Accordingly, experiments were performed on the same sera recorded in Tables V and VII, after three days' storage at 4–5°C. Determinations were made with the same stored plasma which now had a prothrombin time of 80 seconds compared with 61 seconds three days earlier. The three-day-old normal serum lowered the prothrombin time from 80 to 64 seconds, whereas the three-day-old dicumarol serum reduced it to 32. Whether this difference, however, reflected a greater lability, or a lesser initial concentration, of L.F. in the normal serum is not clear.

The oxalated dicumarol serum became inert within three days.

### *Effect of thromboplastin on L.F. of dicumarol serum*

As already shown, thromboplastin added to normal blood reduced serum L.F. activity. On dicumarol blood this effect was not observed. Serum derived from thromboplastin supplemented dicumarol blood contained the same L.F. activity as dicumarol serum derived without the thromboplastin. This was true whether non-oxalated was compared with oxalated serum, and $BaSO_4$ compared with non-$BaSO_4$ serum under the same conditions. It appears, therefore, that the effect of thromboplastin on the L.F. concentration of normal serum was related to the velocity and/or the amount of prothrombin conversion.

Again, in dicumarol-thromboplastin serum L.F. was much less stable in the presence of oxalate.

In these experiments 0.3 ml. of thromboplastin extract was added to 10 ml. of dicumarol blood containing 3 per cent prothrombin. The aged plasma on which the sera were tested had a prothrombin time of 37 seconds.

### *L.F. in hemophilic serum*

Hemophilic serum has more restorative activity on aged plasma than normal serum (Table VIII), despite the fact that the interval between the shedding of the blood and the testing of the serum was

TABLE VIII

*L.F. activity of hemophilic serum (S)*

| Aged plasma* plus: | Proth-T (*sec.*) |
|---|---|
| Exp't 1 | |
| Saline | 80 |
| Norm. S | 26 |
| Hem. S | 17 |
| Norm. $BaSO_4$ S | 34 |
| Hem. $BaSO_4$ S | 28 |
| Exp't 2 | |
| Saline | 80 |
| Hem. S | 14 |
| Hem. $BaSO_4$ S | 28 |
| Hem. Tpl.† S | 38 |
| Hem. Tpl. $BaSO_4$ S | 55 |

* One volume of material added to four of aged plasma. Sera non-oxalated, separated one hour after clotting. $BaSO_4$ S obtained with 50 mgm. $BaSO_4$ per ml.

† One volume of thromboplastin extract to nine of blood.

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[5] Obtained from patients with myocardial infarct treated with dicumarol for the prevention of thromboembolic disease.

inevitably greater as a result of the prolonged clotting time of the hemophilic blood. Also, $BaSO_4$ removes some restorative activity.

As in all the other experiments, oxalated serum from hemophilic blood had far less restorative ability than non-oxalated sera. This was true with whole as well as $BaSO_4$-adsorbed serum.

Supplementing hemophilic blood with thromboplastin, which accelerates coagulation, yields serum containing far less L.F. activity, practically the same as that observed in serum from normal blood similarly supplemented (Table VIII).

### *Serum L.F. in thrombocytopenia and thrombasthenia*

These findings led to experiments in other pathological conditions where prothrombin conversion is reduced as a consequence of inadequate elaboration of thromboplastic material. It is evident (Table IX) that in thrombocytopenic purpura as well as in thrombasthenia, two disorders where prothrombin consumption is poor (15, 27), serum L.F. activity was abnormally elevated. In these cases, the $BaSO_4$ sera also were more potent in rectifying the retarded prothrombin conversion of aged plasma than $BaSO_4$ normal serum. In all instances, the $BaSO_4$ removed the large amounts of residual prothrombic activity characteristically present in serum in these disorders. Recovery in one patient with acute thrombocytopenia was associated with restoration of serum L.F. and prothrombin to normal values.

TABLE IX

*Serum (S) L.F. in thrombocytopenic purpura and thrombasthenia*

Pat. 1—W. Idiopathic thrombocytopenic purpura.
Pat. 2—E. Acute idiopathic thrombocytopenic purpura; quinidine sensitivity.
Pat. 3—G. Easy bruising, ecchymoses, normal to increased bleeding time, increased capillary fragility for years. High residual serum prothrombic activity. Platelet count: 122,000 per mm.$^3$ blood (low normal).

Prothrombin time (*sec.*) of mixture

| Aged plasma* plus: | Thrombocytopenia | | | Thrombasthenia |
|---|---|---|---|---|
| | Pat. 1 | Pat. 2 | | Pat. 3 |
| | *36,000 plts.* | *18,000 plts.* | *130,000 plts.*† | |
| Saline | 66 | 67 | 68 | 76 |
| Norm. Plas. | 16 | 19 | 20 | |
| Pat. Plas. | 16 | 21 | 20 | |
| Norm. S | 31 | 15 | | 21 |
| Pat. S | 16 | 13 | | 18 |
| Norm. $BaSO_4$ S | 37 | 37 | 37 | 61 |
| Pat. $BaSO_4$ S | 23 | 26 | 33 | 50 |

* One volume of material to four volumes of aged plasma.
† After clinical recovery.

### *L.F. in "serum" from afibrinogenemic subject*

Experiments performed on blood from a subject with afibrinogenemia [6] exclude the possibility that L.F. disappears during coagulation as a result of adsorption on the fibrin clot. Blood was drawn with and without added oxalate; the latter remained completely fluid indefinitely. One hour after the blood was shed, oxalate was added to the non-oxalated sample. The bloods were centrifuged. The plasma and "serum" were then incubated at 37°C for 15 minutes. Plasma prothrombin was normal by both one- and two-stage methods. The L.F. activity of the plasma also was normal. The "serum" contained no prothrombin by the two-stage procedure, and the L.F. activity of the $BaSO_4$ adsorbed "serum" was approximately 10 per cent of that of the parent plasma. Also of interest was the finding that the unadsorbed serum contained a normal amount of spca. These observations indicate that the disappearance of L.F. activity during coagulation is not related to formation of a fibrin clot. It may be concluded, furthermore, that spca elaboration is also independent of fibrinogen and its conversion to fibrin.

## DISCUSSION

Although the importance of a non-prothrombin plasma constituent in the conversion of prothrombin by thromboplastin plus calcium is now definitely established, its role in this reaction is obscure. Quick and his collaborators, who have designated the entity by the term "Labile Factor" because of its lability, claim that it enters into the reaction stoichiometrically along with thromboplastin and calcium (28). On the other hand, the Wayne University group (Ware, Seegers, and colleagues), calling their substance "plasma Ac-globulin," consider it to be a relatively inert precursor of "serum Ac-globulin" to which it becomes converted by thrombin. Serum Ac-globulin then functions as an accelerator of the activation of prothrombin (4, 29, 30). Owren, who holds similar views, terms his substance Factor V which is converted to Factor VI during coagulation, and which accelerates thrombin elaboration (12, 5).

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[6] We are indebted to Dr. Louis K. Diamond of the Children's Medical Center, Boston, for making blood, from this patient, available.

Regardless of whether these moieties are identical (and it is becoming increasingly evident that they are), certain facts are clear. When L.F. is progressively reduced below 50 per cent of that in normal plasma, the prothrombin time increases (8). Prothrombin consumption is also curtailed (31). Thus, a *given prothrombin time reflects* the amount of L.F. (or plasma Ac-globulin and Factor V, if we assume that they are identical) as well as the concentration of prothrombin itself. Accordingly, in order to measure prothrombin as such, adequate quantities of this other factor must be assured. This point has received scant consideration in reports on serum prothrombin and on prothrombin consumption during coagulation, especially those based upon one-stage prothrombin determinations, where the velocity of prothrombin conversion is measured.

The foregoing data, in accordance with recent reports (28, 32), show that substantial amounts of L.F. disappear during normal coagulation. Depletion of L.F. begins before prothrombin falls off, but soon the rates with which both are consumed seem parallel. Accelerating and increasing prothrombin conversion with supplements of thromboplastin causes greater losses. Conversely, in hemophilia, thrombocytopenia, and thrombasthenia where prothrombin conversion is retarded or reduced, L.F. consumption appears reduced. Furthermore, curtailing the amount of available prothrombin also decreases the amount of L.F. consumed.

Accelerating coagulation and increasing prothrombin consumption in hemophilia by additions of thromboplastin induces greater L.F. consumption. It may, therefore, be concluded that this clotting factor is utilized in relation to the amount and/or velocity of prothrombin consumption. Furthermore, critically curtailing the concentration of L.F. limits the velocity as well as the amount of thrombin evolution (33). These findings lend support to the idea that L.F. enters into the reaction as a real participant rather than the alternative view that it is merely an "accelerator" acting outside the thrombin-forming system proper. This is in accordance with the interpretation of Quick and Stefanini (28) regarding L.F. and Lewis and Ferguson concerning Ac-globulin (34).

The amount of L.F. in serum is considerably influenced by the presence of decalcifying anticoagulants and the interval of time elapsing after coagulation. These facts must be considered in studies where these agents may be added to blood at various intervals after blood is shed in order to stop prothrombin conversion at various stages of the coagulation process. It is noteworthy that L.F. deteriorates more rapidly in oxalated than in citrated serum, as in plasma under the same condition.

All of these facts are pertinent to the determination of serum prothrombin and prothrombin consumption by the one-stage method. The orthodox procedure (18), modified by the addition of fibrinogen, will give grossly inaccurate results for two reasons: (*a*) During coagulation the L.F. disappears to a variable extent, which is considerably influenced by conditions of coagulation. Consumption of L.F. may reduce its concentration in serum to critically low levels, thus causing *elevated* prothrombin times. (*b*) Also, the prothrombin conversion accelerator (spca) which *evolves* during coagulation varies in its concentration in serum, again depending upon various conditions of coagulation (23). This clot-promoting factor increases the velocity of prothrombin conversion by thromboplastin and calcium, resulting in *shorter* prothrombin times than can be attributed to the prothrombin itself. It has already been shown that for this reason, one-stage determinations of the prothrombin in serum are inaccurate (15). Now there is additional basis for this conclusion.

Difficulties also arise in the determination of serum L.F. by the one-stage procedure. Clearly, the substance is measurable by its restorative activity on aged plasma in which this clotting factor has largely deteriorated. Thus, the restorative effect of serum might validly be considered a measure of L.F. if we could be sure that during coagulation alterations did not take place in components *other* than prothrombin or L.F., which might influence the velocity of prothrombin conversion in aged plasma. That such alterations do occur, however, has been shown conclusively. Spca evolves which, in the presence of L.F., will accelerate thrombin evolution in stored plasma (24). Seegers and his group had shown earlier that serum and serum fractions activate and accelerate prothrombin conversion to a greater degree than plasma, and Owren has reported similar observations (12). Also, Milstone found that in the course of coagulation, "prothrombokinase" is converted to "thrombokinase" which is a potent accelerator of prothrombin conversion (35).

It is accordingly evident that some consideration must be given to clot-promoting agents which *arise* as a consequence of the coagulation process, and which may accelerate prothrombin conversion in aged plasma, simulating or enhancing L.F. activity. This is substantiated by the observations that $BaSO_4$ removes considerable restorative activity from serum despite the fact that this agent does not appreciably adsorb L.F. Since $BaSO_4$ removes spca (23) and thrombokinase (35), the effect on the restorative activity of serum is attributed to removal of this (these) substance(s). It should also be noted that $BaSO_4$ also adsorbs residual serum prothrombin. The restorative activity of $BaSO_4$ treated serum is, therefore, a more accurate index of serum L.F. than untreated serum.

The question arises whether serum Ac-globulin and Factor VI can be similarly excluded. According to Murphy and Seegers (36) and as has been confirmed by us (24), serum Ac-globulin is extremely unstable in normal human serum, virtually disappearing shortly after coagulation. The same is said to be true of Factor VI (12). This does not necessarily obviate the need for considering these factors because under abnormal conditions of coagulation they may be more stable, persisting in the serum. Indeed, substantial Ac-globulin activity is demonstrable in human serum prepared from blood allowed to clot slowly in siliconized tubes (36).

In a previous paper (24) evidence was presented which indicated that "serum" Ac-globulin probably comprises a mixture of plasma Ac-globulin (L.F.) plus spca, which together account for its greater prothrombin-converting activity than plasma Ac-globulin alone. The relatively low initial concentration of Ac-globulin in human plasma and its rapid depletion during coagulation explains the apparent extreme lability of "serum" Ac-globulin in human, in contrast to bovine, serum. On the basis of this interpretation, and assuming Factor VI to be identical with "serum" Ac-globulin, removal of spca by adsorption with $BaSO_4$ adequately takes care of clot-promoting activity of serum over and above that of its parent plasma as far as the restorative effect of serum on aged plasma is concerned.

In evaluating the restorative effect of serum as a measure of its L.F. content, consideration should also be given to the products of platelet disintegration which are said to accelerate prothrombin conversion in aged plasma (37, 38). It appears that platelets contain at least two clot-promoting substances, one which resembles Ac-globulin, and another which accelerates the interaction of thrombin with fibrinogen (39, 30).[7] Whether these appear, and persist, in serum as a consequence of clotting is not clear. Pending further investigation, it may be concluded that the restorative effect of $BaSO_4$ serum on aged plasma provides a suitable method for measuring serum L.F. By comparing the prothrombin times of mixtures containing various dilutions of $BaSO_4$ serum in aged plasma with similar mixtures containing the $BaSO_4$ parent plasma, the L.F. of the serum in per cent of that originally present in the blood can be determined.

## CONCLUSIONS

1. Normal serum has as much, and frequently more, restorative activity than the parent plasma on the retarded prothrombin conversion of aged plasma.

2. This is largely due to spca which evolves during coagulation and which, together with whatever Labile Factor remains unconsumed, accelerates prothrombin conversion.

3. Removal of spca by adsorption with $BaSO_4$ yields a serum which still has some restorative activity. This activity is a measure of residual L.F. in serum, which under normal conditions is small.

4. L.F. begins to disappear from blood shortly after its withdrawal, even before detectable changes in the prothrombin concentration have occurred. Within 40 minutes consumption of both factors is virtually complete. Disappearance of L.F. during coagulation is unrelated to the conversion of fibrinogen to fibrin.

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[7] It has previously been shown that spca does not act on this phase of the coagulation process (22, 24).

5. Accelerating coagulation and prothrombin conversion by thromboplastin supplements increases the amount of L.F. consumed.

6. Conversely, curtailing the amount of prothrombin converted to thrombin, by dicumarol or by pathological conditions such as hemophilia, thrombocytopenia or thrombasthenia results in sera containing abnormally large amounts of L.F. This could be corrected in hemophilia by thromboplastin supplements, but not in dicumarol blood.

7. These observations lend support to the concept that L.F. is an active participant in the prothrombin conversion reaction.

8. Serum L.F. seems more stable in citrate than in oxalate, but not as stable as in native serum. This is in accordance with its behavior in plasma under similar conditions.

9. These findings are discussed insofar as they relate to methods for the determination of serum prothrombin, serum L.F. and prothrombin consumption under normal and pathological conditions.

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## ASSOCIATION ANNOUNCEMENT

The 43rd annual meeting of the American Society for Clinical Investigation will be held at the Chalfonte-Haddon Hall, Atlantic City, N. J., on Monday, April 30, 1951, at 9 a.m.

Other meetings to be held also at the Chalfonte-Haddon Hall are:

The 8th annual meeting of the American Psychosomatic Society, Satuday, April 28, 1951.

The National Meeting of the American Federation for Clinical Research, Tuesday, May 1, 1951.

The 64th meeting of the Association of American Physicians, Tuesday, May 1, and Wednesday, May 2, 1951.

# ABSORPTION STUDIES USING PORTAL ANASTOMOTIC VEINS[1]

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(Submitted October 13, 1950; accepted, December 11, 1950)

In man the portal circulation, being without major and macroscopic connection with systemic veins, has been hidden from the clinical investigator. Even the intravascular catheter has not invaded the duplex circulations feeding the liver, but only its drainage system. Thus circulatory inaccessability of the key digestive gland in man has left it mysterious, a metabolic sphinx. Surgeons, deleting the spleen from persons with portal hypertension, have recorded sporadic data on portal pressure and blood gases (1, 2). Physiologists have estimated total hepatic blood flow in man by the bromsulphalein clearance test (3) which permits at least reasonable approximations. Sherlock and Walshe (4) added some facts by measuring portal blood from large superficial anastomotic veins in a single patient with cirrhosis. They reported observations in a patient with a large abdominal vein apparently draining blood from the upper segment of the alimentary canal into the right great saphenous vein. Their attention was concentrated on a comparison of systemic and anastomotic blood levels of glucose, galactose, fructose, fat and nitrogen at appropriate intervals after ingestion of measured quantities of these substances or their precursors, singly or in combination.

The ordinary collateral veins over the abdomen of cirrhotic patients have been studied in an effort to determine portal pressures (1, 5) and circulation time (6). Billings and DePree suggested that comparison of blood from the abdominal collaterals and systemic veins following administration of glucose orally might permit the differentiation of cirrhosis from other disorders giving rise to collateral vessels over the belly (7).

Myers (8) has made an effort at physiologic partition of hepatic arterial and portal venous components of the total blood flow to the liver by measuring the gas content of arterial, hepatic vein and caput medusae blood. If one assumes that there has been no reflux from hepatic artery into the portal anastomotic vessel and that the cases were not indeed instances of the Cruveilhier-Baumgarten syndrome (9), such observations may bring crucial evidence to impinge on Dock's surprising observations on postmortem infusion of hepatic artery and portal vein with kerosene (10).

We are reporting studies on two patients (see Appendix) with cirrhosis who had portal anastomotic veins large enough to permit multiple studies of absorption. One of these patients died and postmortem examination revealed an unusual connection between a branch of the superior mesenteric vein and the superficial abdominal vein which coursed upward and emptied into the superior vena cava via the left internal mammary and innominate veins. Details of the connections are given in Figures 9 and 10. Our observations were restricted in part by the chemical facilities at hand when the two patients were under observation almost simultaneously on the wards. By the time we had prepared to do more elaborate tests, one patient died in the hospital and the other went home and died. No others with such vessels have been found in our last 20 cirrhotic patients. Therefore, we are presenting our data as modest increments to knowledge which must be extended before we draw general conclusions.

## METHODS

Our observations were made by comparing the levels of constituents of blood samples taken simultaneously from the antecubital and portal anastomotic vein at appropriate intervals after the ingestion of the mixture whose composition is listed in Table I. Studies included those on glucose (11), fructose (12) and vitamin A (13,

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[1] Aided in part by the Medical Trust Fund of the Department of Internal Medicine, and research grants from the Division of Research Grants and Fellowships of the National Institutes of Health, U. S. Public Health Service, and the Commission on Liver Disease of the Armed Forces Epidemiological Board.

TABLE I

*Contents of test meal*

60 grams of protein (skim milk and skim milk powder)
80 grams of fat (cream)
50 grams of glucose
50 grams of fructose
500 mg. of ascorbic acid
5 mg. of thiamine
10,000 units vitamin A (M. W.)
25,000 units vitamin A (N. N.)
250 ml. water

14), neutral fat (15), thiamine (16, 17), ascorbic acid (18), amino acid nitrogen (19) and urea nitrogen by the urease method. In no case was the error of the method more than ± 5%. We did all tests in duplicate except for glucose and urea nitrogen. The amount of blood which could be withdrawn from the abdominal vein sometimes limited the number of tests it was possible to do. Some samples were used for tests which would have been omitted had we been able to predict results. We collected further data on oxygen saturation before and after eating; on circulation time and venous pressure. We did the absorption studies the day following paracentesis in order to reduce by some degree the mechanical interference with alimentary function and to make the abdominal vein more accessible. For a period of one week prior to each absorption study, both patients were fed a high-carbohydrate, high-protein and unrestricted-fat diet. Supplemental vitamins were furnished in the form of multi-vitamin capsules and crude brewer's yeast. In addition, subject M. W. received thiamine, riboflavin and vitamin K.

## OBSERVATIONS

The information collected in this study is presented in a series of charts.

### *Carbohydrate*

Blood samples were taken after a 14-hour fast, and 50 grams of glucose were given orally at zero time. From Figure 1 the following facts are noted.

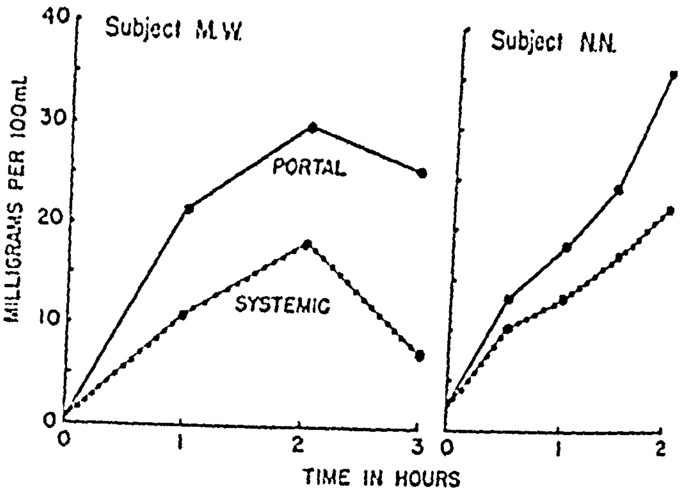

FIG. 2. COMPARISON OF PORTAL AND SYSTEMIC BLOOD FRUCTOSE LEVELS FOLLOWING INGESTION OF TEST MEAL

(*1*) The fasting level of glucose in abdominal and antecubital vein was the same. (*2*) In all tests the level of glucose rose more rapidly, and for 1½ to two hours was sustained consistently at higher levels in the abdominal vein than in the vein of the arm. In the first test in subject M. W. the level of glucose in the abdominal vein and arm vein was elevated above the normal at the two-hour period. In the second test the abdominal vein blood showed higher glucose values at the end of two hours with the levels both approaching normal at the end of three hours. The third test three months later when the patient was clinically much worse showed a high glucose level in the abdominal vein blood at the end of two hours. No later determinations were made. (*3*) In subject N. N. the arm and abdominal blood glucose levels were slightly elevated with the abdominal blood level being higher at the end of two hours. There was no regularity of time relations for peak of glucose

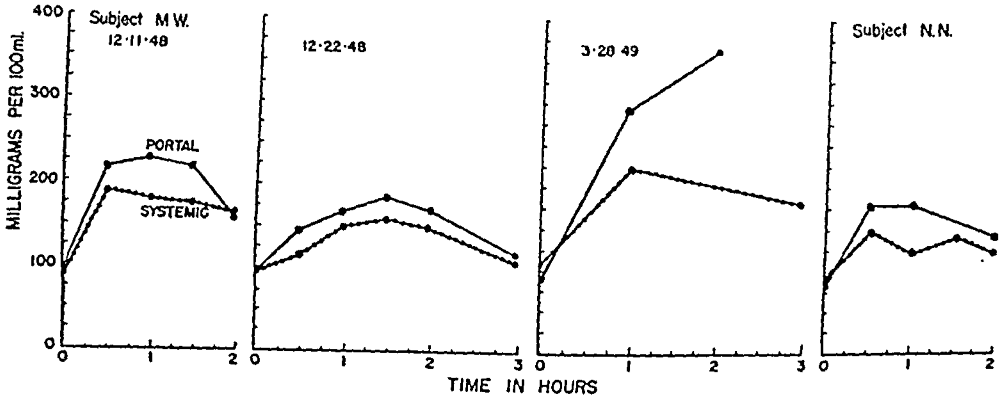

FIG. 1. COMPARISON OF PORTAL AND SYSTEMIC BLOOD GLUCOSE LEVELS FOLLOWING INGESTION OF TEST MEAL

in the abdominal vein, although the average time for peak elevation was somewhat later (75 min.) than for the arm vein (53 min.). The peaks in all four tests differed varying from one-half to two hours. The fasting blood glucose levels were normal in both subjects. (*4*) Fructose (Figure 2)—Both subjects had normal fasting levels and a decreased tolerance. The absolute value of the blood fructose was less than that of glucose although proportionately the rise of fructose was greater than that of glucose. Except for the fasting samples, the fructose levels in the abdominal vein were higher at all times in both subjects. Peak level in abdominal and arm vein blood occurred at the end of two hours. No later determinations were carried out on subject N. N.

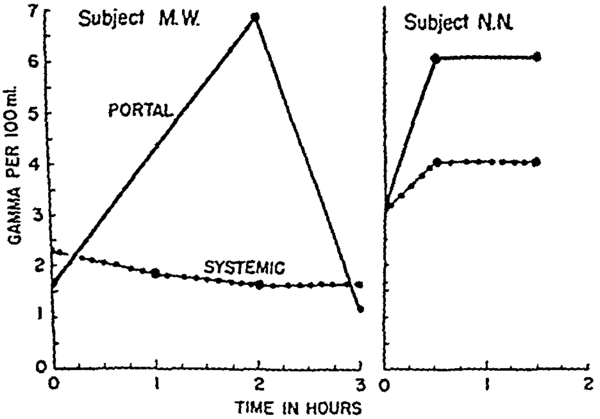

FIG. 3. COMPARISON OF PORTAL AND SYSTEMIC BLOOD THIAMINE LEVELS FOLLOWING INGESTION OF TEST MEAL

### *Water Soluble Vitamins*

Five mg. of thiamine were given orally as the test dose. There was no clinical clue to specific thiamine deficit but the dietary history suggested that there was moderate thiamine undernutrition.

From Figure 3 it may be seen that: (*1*) Anastomotic vein blood rose to much higher levels than did blood from the arm vein. (*2*) Rise in systemic vein was absent or very slight. (*3*) The curves do not have enough points to describe a general pattern of behavior. (*4*) In one subject the thiamine level of anastomotic vein blood had returned to the control level at three hours.

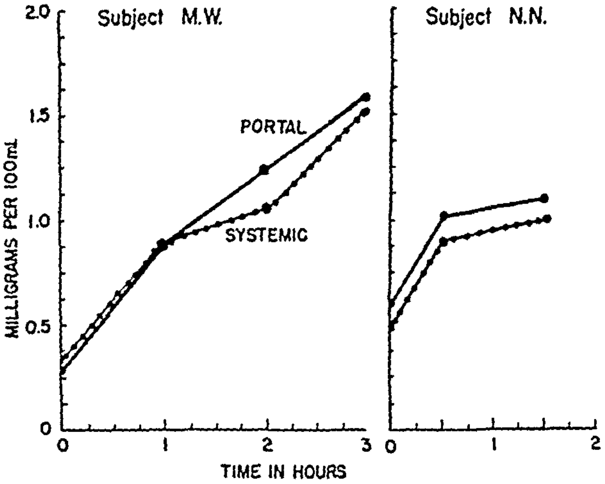

FIG. 4. COMPARISON OF PORTAL AND SYSTEMIC BLOOD ASCORBIC ACID LEVELS FOLLOWING INGESTION OF TEST MEAL

Ascorbic acid was given orally in the amount of 500 mg. Figure 4 reveals that: (*1*) There was no substantial difference in the levels from portal and systemic blood in fasting or postprandial samples. (*2*) Portal blood was regularly higher than systemic blood, though not to a significant degree. (*3*) No fall in level had occurred at the end of two or three hours in the two cases.

### *Vitamin A*

Vitamin A was given orally, 10,000 units, to subject M. W. and 25,000 units to subject N. N. Figure 5 reveals: (*1*) Similar fasting levels in portal and systemic blood. (*2*) More rapid and higher rises in portal than in systemic blood. (*3*) Return to control values at three hours in one subject.

### *Neutral Fat*

Neutral fat was given as 80 grams of cream. Figure 6 indicates: (*1*) A distinctly higher systemic than portal fasting level. (*2*) Irregular

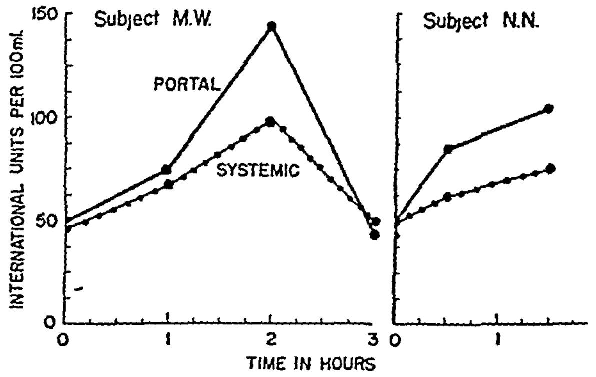

FIG. 5. COMPARISON OF PORTAL AND SYSTEMIC BLOOD VITAMIN A LEVELS FOLLOWING INGESTION OF TEST MEAL

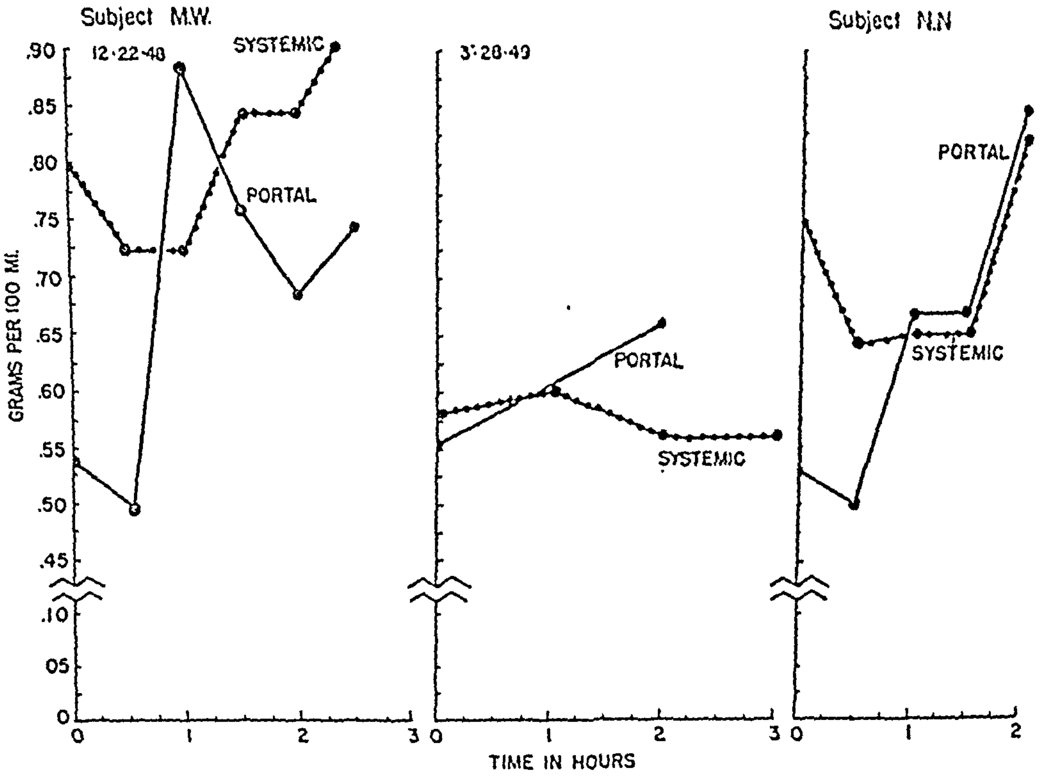

Fig. 6. Comparison of Portal and Systemic Blood Plasma Lipid Levels following Ingestion of Test Meal

rises in which portal blood levels rose at a more rapid rate. (*3*) Two of the three tests indicate a delayed rise of lipid in systemic blood.

### *Nitrogen*

Sixty grams of protein in the form of skim milk and skim milk powder were given. Figure 7 for amino acid nitrogen indicates that: (*1*) The tests showed no clear difference in portal and anastomotic vein levels of amino acid nitrogen though there was a tendency for a peak to occur at two to 2½ hours. (*2*) The levels varied erratically.

Figure 8, indicating blood urea nitrogen, shows: (*1*) Similar fasting levels in portal and systemic blood; (*2*) a rise in systemic blood urea nitrogen at one hour, in one instance falling rapidly and in the other sustained for some time.

### *Circulation Time*

Circulation time was determined by the decholin method. The results are in Table II. The

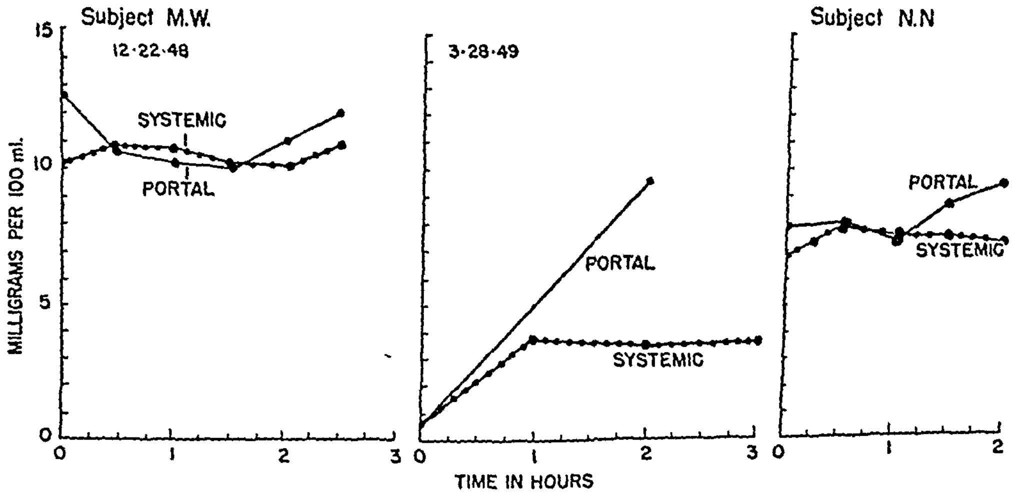

Fig. 7. Comparison of Portal and Systemic Blood Amino Acid Levels following Ingestion of Test Meal

blood from the abdominal vein reached the tongue in 11 and 14 seconds, distinctly less than the arm to tongue time. Femoral vein to tongue time was at the upper range of normal.

### *Venous Pressure*

The determinations of portal vein pressure are compared with those from the antecubital and femoral vein in Table III. The right atrium was the standard zero reference.

In one subject (N. N.) studies of blood gases were attempted in the fasting state and during absorption. The necessity of producing stasis in order to obtain blood from the portal vein, which was copied as far as possible in taking blood from the arm, makes the data presented in Table IV of questionable accuracy. The blood from the portal anastomotic vein was regularly of a brighter red color than that from the arm vein.

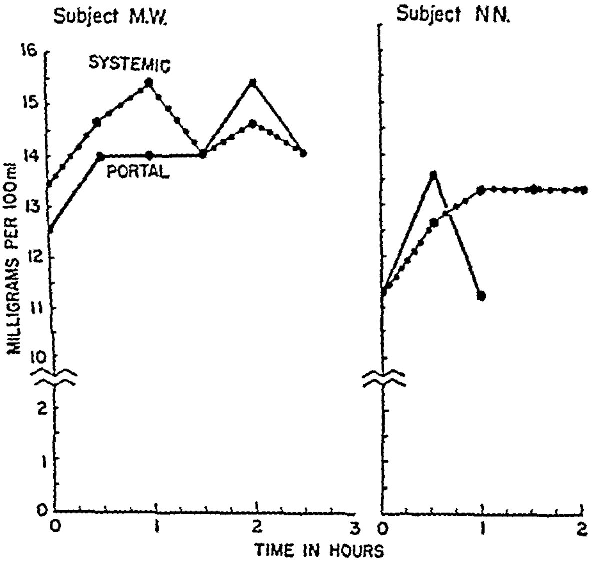

FIG. 8. COMPARISON OF PORTAL AND SYSTEMIC BLOOD UREA NITROGEN LEVELS FOLLOWING INGESTION OF TEST MEAL

TABLE II

*Circulation time in seconds (decholin)*

| | M. W. | N. N. |
|---|---|---|
| Anastomotic vein to tongue | 11* | 14 |
| Arm to tongue | 16 | 17 |
| Femoral vein to tongue | 26 | 27 |

* Each number is the average of two determinations.

DISCUSSION

Although studies of absorption were made by analysis of portal vein blood in animals (20) in 1877 no similar efforts to evaluate the phenomena of absorption in man have been made until recently. While the results of the biochemical tests on various substances are roughly comparable in the two subjects, and postmortem observations in one of them established the anatomical relations of the portal anastomotic vein, the data are scarcely adequate for broad generalization. Together with the patient reported by Sherlock and Walshe (4) whose anastomotic vein carried blood down to the saphenous vein, three subjects with large portal anastomotic veins have been investigated. Our patients both had a similar pattern of superficial venous drainage in a cephalic direction by a large anastomotic vein. This was demonstrated by local pressure which indicated that the vein filled more rapidly from below than from above; and the exaggerated prominence produced by the Valsalva procedure. Because we

TABLE III

*Venous pressures (cm. water) referred to right atrium as zero point*

| | M. W. | N. N. |
|---|---|---|
| Arm | 11* | 9 |
| Arm (after R.U.Q. pressure) | 12.5 | 9 |
| Femoral | 11.5 | 9 |
| Femoral (after R.U.Q. pressure) | 19 | 13 |
| Anastomotic vein | 22 | 19 |
| Anastomotic vein (after R.U.Q. pressure) | 23 | 19 |
| Anastomotic vein (after Valsalva procedure) | 26 | 25 |

* Each number is the average of two determinations.

TABLE IV

*Oxygen saturation (subject N. N.)*

| | Volumes % | Saturation (%) |
|---|---|---|
| *Fasting Blood* | | |
| Arm vein | 7.0 | 38.8 |
| Anastomotic vein | 14.0 | 82.5 |
| After Meal | | |
| Arm vein | 5.0 | 27.8 |
| Anastomotic vein | 14.2 | 79.0 |
| Oxygen capacity | 18.0 | |

could not estimate the moiety of portal blood carried in this vein, it is idle to speculate upon the quantitative aspects of the data. They merely indicate trends. Another limitation in the study is the reliability of the venous blood from the arm, representing not mixed venous blood but a particular sample. In essence we are comparing the constituents of two different effluent streams of blood, but we cannot relate the observed data to total quantities. The effect of the water of the test feeding added another variable.

A number of factors militate against the application of our findings to the normal processes of absorption in man. The metabolic function of the cirrhotic liver is impaired, but no single test or clinical finding permits precise quantitation of the several disturbances which would influence absorption and hepatic metabolism. The higher pressure of blood in the portal circuit well known clinically, demonstrated at operation (1, 21) and measured directly in our subjects might be expected to diminish or retard absorption from the gut. It is not known to what extent commingling of arterial blood from the hepatic artery with venous blood from the intestine occurs, or even that it has any quantitative significance. Our observations, therefore, though they provoke speculation, provide no conclusive solution to the problems they bear upon. Because of the anatomical relations in subject M. W., it is probable that significant admixture with systemic blood did not occur.

*Carbohydrate.* It is the consensus of investigators that glucose is absorbed by a phosphorylation mechanism with hexose phosphoric acid serving to transport glucose across the mucosa where glucose again is formed and transported as such to the liver. Our studies (Figure 1) suggest that this process is protracted over periods of at least two hours. In all observations the curves of the systemic glucose and fructose blood levels are typical of those found in liver disease, namely, normal fasting levels with decreased tolerance after ingestion of the sugar. The elevated levels are attributed to impairment of the liver's function in carbohydrate regulation. The prolonged elevation likewise may result from hepatic malfunction and probably impaired intestinal absorption as well. The earlier and higher rise and the more rapid return to normal of glucose in contrast to fructose is in keeping with the fact that gastro-intestinal absorption of glucose is more rapid than that of fructose although the rates of utilization in the liver are similar (22). The factors of tissue utilization of glucose, possible glucogenesis from the protein in the test feeding, and the effect of fat in delaying absorption could not be appraised.

*Water soluble vitamins.* Thiamine levels in portal blood rose rapidly whereas the rise in systemic blood was meagre or did not follow (Figure 3). Perhaps this merely reflects thiamine depletion in the tissues. We have no knowledge of what metabolic alterations were produced because of the bypass of so much portal blood to the general systemic circulation before passage through the liver. The fact that portal blood levels rose without a similar rise of systemic blood thiamine raises a question about the usual interpretation of such flat tolerance curves as indicative of reduced absorption; and it must be concluded that thiamine was utilized, stored, destroyed, or excreted rapidly.

Ascorbic acid levels followed a different course; and there was no substantial difference between portal and systemic blood samples. One could interpret this as an indication of a state of saturation, though the initial levels are below the usually accepted normal range. Another assumption might be that the use or elimination of ascorbic acid was diminished.

*Fat soluble vitamins.* To some extent the curves of vitamin A levels (Figure 5) were similar to those for glucose. Subject N. N., who received 25,000 units, had less of a rise than subject M. W., who received 10,000 units though the time intervals of sampling and the duration of the tests differed.

*Lipids.* There was a sharp disparity between portal and systemic blood levels for neutral fat in the fasting state, suggesting that the liver was liberating fat into the blood stream, or possibly that it was removed somewhere in the long circuit between hepatic vein and the portal collecting system. In two instances there was an initial fall and then a sharp rise in portal blood to levels somewhat higher than the simultaneous systemic blood level.

*Nitrogen.* Figures 7 and 8 exhibit data from studies on portal and systemic blood urea nitrogen and amino acid nitrogen. Similar levels for amino acid nitrogen occurred during the early stages of absorption and not until the second hour was the level in portal blood significantly higher than in systemic vein blood at a time when the breakdown of protein to amino acids in the gut was well advanced. The curves for urea nitrogen (Figure 8) suggest several interpretations. In M. W. it may be supposed that the formation of urea from amino acid was advancing rapidly and reached an early peak, after which the process declined or excretion increased. In N. N. the curves may be taken to suggest the absorption of urea, or its formation in the gut, though the differences are not striking, and the variations suggest that the results may be in error.

*Oxygen.* It is probable that technical difficulties with the collection of blood from the anastomotic vein, the necessity for using pressure and stasis and efforts to duplicate the situation in the vein of the arm make the quantities unreliable. Nevertheless, in each sample taken from the abdominal vein it was obvious that the blood had the bright red color nearly that of arterial blood. By ordinary inspection, and by the use of red goggles, the anastomotic vein appeared less dark than neighboring veins coursing up over the abdomen. It failed to show up as well as we expected with infrared photography. We have demonstrated in some other patients with cirrhosis identical blood glucose levels in arm and abdominal collateral veins after a test meal, and in spite of the report of Billings and DePree (7), we are of the opinion that some of the prominent abdominal veins in cirrhotics carry unmixed systemic blood from the lower extremity. The high oxygen content of the anastomotic vein may indicate (*1*) a reflux of hepatic artery blood from the liver, (*2*) an open state of the numerous arteriovenous connections in the gut, or (*3*) very modest oxygen consumption by the gut during absorption. Whether or not these speculations are correct or some other cause exists, the finding casts a reasonable doubt on estimates of the moiety of blood supplied to the liver by hepatic artery and portal vein where gauged by gases from portal vein blood (8). The very low levels of oxygen in systemic venous blood are puzzling although reduced oxygen content of the blood has been reported in cirrhotics (23).

*Venous pressure.* The dynamic state of the portal circulation, a low pressure system suspended between two capillary beds and thus indeed a veritable *rete mirabile*, has been well hidden. Our only information in man has come from surgeons who have measured pressure directly at laparotomy, usually in subjects with portal hypertension. Observations on abdominal veins by Levy and Burch (5) give no indication that portal anastomotic veins were used and the technique of occlusion used by Davidson, Gibbons and Faloon (24), while indicating the pressure which may be reached, introduces an artifact if any appreciable quantity of blood flow is obstructed. Our observations were carried out the day after abdominal paracentesis, when there was relatively little distention, even though some ascitic fluid remained. The portal pressure of 22 and 19 cm. of water in the two subjects was significantly higher than the systemic venous pressure (Table III). The Valsalva maneuver and hepatic jugular reflux test produced the expected changes.

Circulation time studies (Table II) suggest that blood from the anastomotic vein traversed a shorter distance or moved more rapidly than that from the arm.

## RESUMÉ

The data on absorption and portal circulation gained from two subjects with portal anastomotic veins who were fed a mixture of test substances permit the following tentative statements:

1. Carbohydrate was absorbed rapidly from the upper part of the alimentary canal and the high level was sustained 90 minutes or more.
2. Thiamine, though it reached high levels in portal blood, did not rise significantly in systemic venous blood.
3. Ascorbic acid levels did not differ in portal and systemic blood during the rise following absorption.
4. Vitamin A was absorbed rapidly from the upper alimentary canal and systemic blood levels rose more slowly and not to the same degree.
5. The fasting level of lipids initially was lower in portal blood than in blood from the arm vein. In two instances there was an early fall followed by a sharp rise.
6. Amino acid rise in portal vein was delayed, variable and of small degree. Urea nitrogen was higher in systemic than portal blood during much of the absorption period, but the erratic behavior precludes analysis.

TABLE V

| Subject | M. W. | M. W. | M. W. | N. N. |
|---|---|---|---|---|
| | *12-11-48* | *12-22-48* | *3-28-49* | *6-28-49* |
| R.B.C. | 4.2 | 4.0 | 4.9 | 4.0 |
| Hg. (*gm. per 100 ml.*) | 13 | 13 | 14 | 11.9 |
| W.B.C. | 7,000 | 6,300 | 9,100 | 11,690 |
| Total protein (*gm. per 100 ml.*) | 5.4 | 6.5 | 6.2 | 6.4 |
| Albumin (*gm. per 100 ml.*) | 2.5 | 3.0 | 2.9 | 3.2 |
| Globulin (*gm. per 100 ml.*) | 2.9 | 3.5 | 3.3 | 3.1 |
| Urine bilirubin | 0 | 0 | 0-3+ | 4+ |
| Urine urobilinogen | 4+ | 4+ | 4+ | 1+ |
| Van den Bergh (*1 min. mg. per 100 ml.*) | — | 0.5 | — | — |
| Van den Bergh (*30 min. mg. per 100 ml.*) | 3.3 | 2.7 | 1.8 | 6.4 |
| Alk. phosphatase (*Bod. units*) | 5.2 | 6.4 | 8.4 | 17.2 |
| Ceph. flocculation | 4+ | 3+ | 2+ | 1+ |
| Thymol turbidity units | 22 | 26 | 27 | 2.6 |
| Zinc sulfate turbidity units | — | 25 | 27 | 11.0 |
| Cholesterol (*mg. per 100 ml.*) | 187 | 233 | 324 | 168 |
| Cholesterol esters (*mg. per 100 ml.*) | 83 | 152 | 219 | 75 |
| Prothrombin time (*sec.*) | 46 | — | 44 | 39 |
| Prothrombin time (*sec.*) (control) | 36.3 | — | 41.7 | 29 |
| Bromsulphalein retention (*5 mg. per kilo.—45 minutes*) | 44% | — | 29% | 42% |
| 17-Ketosteroids (*mg. per 24 hr.*) | — | — | 2.2 | 1.4 |

7. Oxygen content of portal vein blood was elevated, portal venous pressure was higher than systemic pressure and circulation time from anastomotic vein to tongue shorter than from arm to tongue.

8. These observations leave unanswered more questions than they illuminate. They emphasize a clinical finding first used by Sherlock and Walshe to solve some of the metabolic problems concealed in the hitherto inaccessible reaches of the portal blood system in man.

## APPENDIX

### *Case Reports*

*M. W.*, a 43 year old railroad station agent, had three admissions to the Medical Service of the University Hospitals: 8 November—30 December, 1938; 21–30 March, 1949; and 30 September—5 October, 1949. His story on the first admission included a bout of "yellow jaundice" in 1932 and no recent but probably previous excessive use of alcohol. His illness began insidiously in March 1948, with discomfort in both flanks, indigestion, sporadic nausea and vomiting. Within two months his belly became swollen, his appetite declined and food gave rise to a sensation of bloating. Though there was weakness, malaise and easy fatigability, his weight rose from 155 to 162 pounds. Hemorrhoids appeared during the month before admission. He had become aware of the advent of vascular spiders within six weeks of the onset of his illness. During the month before admission his ankles became swollen, especially in the evening. Prior to his illness his diet was adequate. Nothing else of consequence was found in his past history or in the family history.

Examination disclosed a well-built, undernourished man without jaundice. He was alert, cooperative and in no acute distress. His skin had a slatey gray cast and was liberally sprinkled with vascular spiders though there was no palmar erythema or gynecomastia. Pectoral,

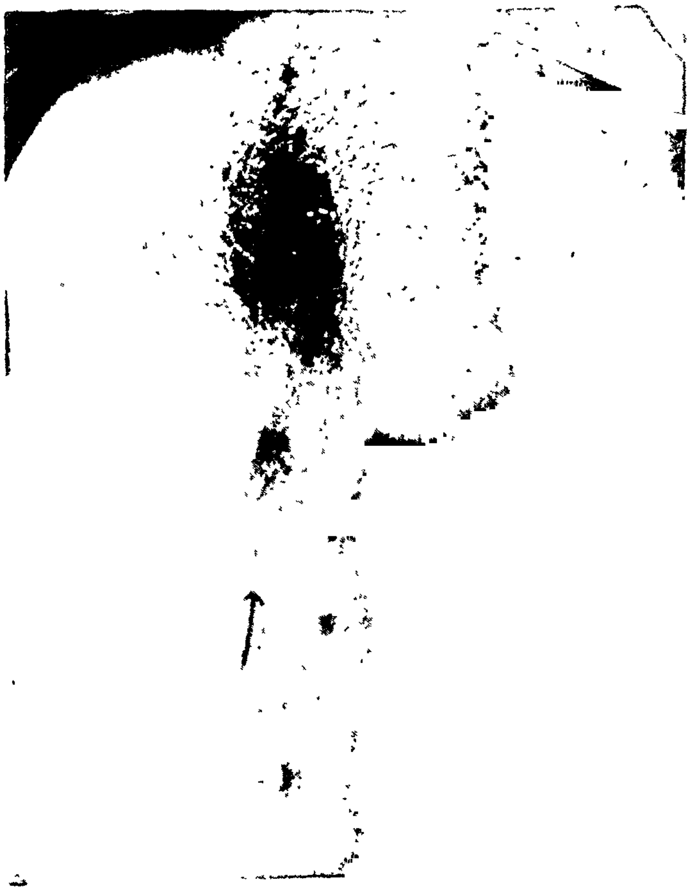

FIG. 9a. PHOTOGRAPH OF ANTERIOR ABDOMINAL WALL VEIN IN PATIENT M. W. DURING A VALSALVA PROCEDURE
Arrow marks direction of blood flow.

pubic and axillary hair was ample. A few moist rales were heard at the right base posteriorly where impairment of the percussion note was found. The diaphragms were elevated and the abdomen was much distended. A fluid wave was found. There were a number of dilated veins coursing upward over the abdomen; and a very large one was found emerging in the midline to follow a tortuous course upward to the region of the xiphoid where it disappeared below the rib margin to the right. The Valsalva procedure caused it to stand out elegantly in relief (see Figure 9a). After paracentesis of 6 liters the spleen was felt, but not the liver. There were external hemorrhoids. Other features of the examination were not unusual.

*Course:* During the first admission he was kept in bed and given a diet high in protein and carbohydrate with supplemental vitamins. Salt was restricted and mercurial diuretics were used repeatedly. Five paracenteses, each yielding 5 to 6 liters, were done between 11 November and 28 December.

There was gradual though not very substantial improvement and he was sent home on the same regimen where he required abdominal paracentesis at about ten-day intervals.

*Second admission:* The physical signs had not changed appreciably. Laboratory data are included in Table V. Two paracenteses were done yielding 2 liters each and absorption studies were carried out. He was discharged on the same regimen as before.

*Interval:* During his stay at home he continued to become weaker but adhered to his diet and medication, needing paracentesis at weekly intervals. A few days before his final admission he suddenly became stuporous. Bright red blood was passed by rectum several times.

*Final admission:* It was suspected that some of the lethargy might have resulted from barbiturates which he had been given just prior to hospitalization. He became alert enough to eat after 4 liters of slightly turbid ascitic fluid were removed, but soon lapsed back into stupor. He was then sustained by intravenous glucose and

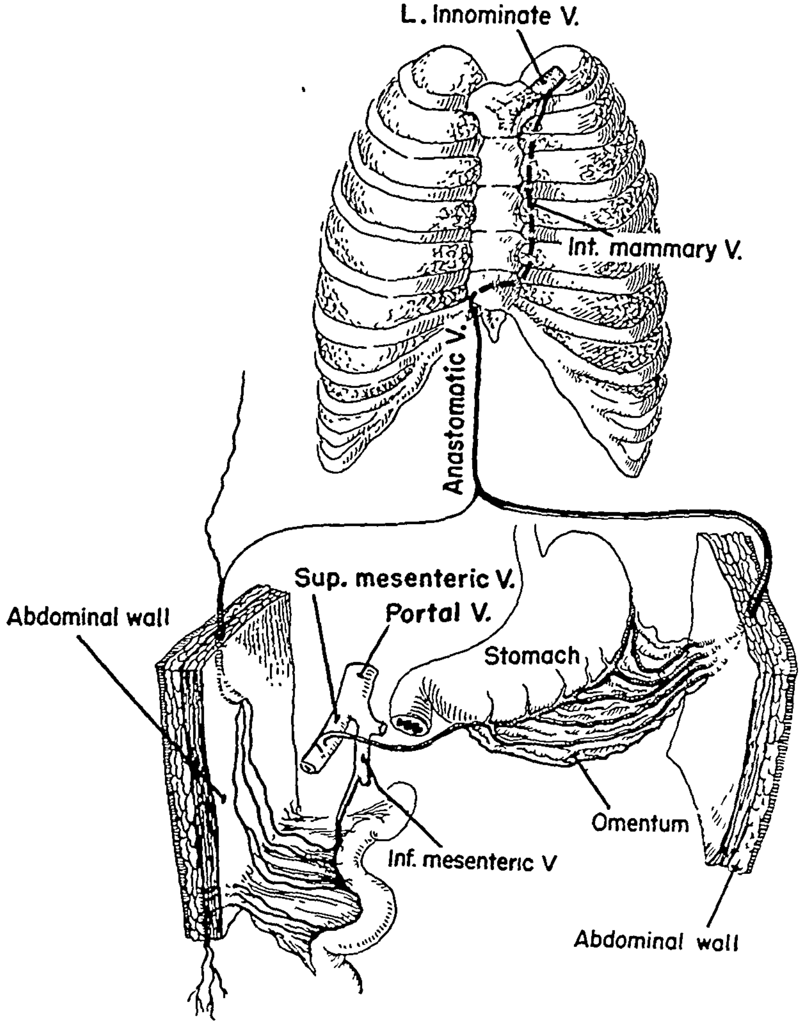

Fig. 9b. Diagram Illustrating Origin and Course of Abdominal Anastomotic Vein in Patient M. W.

salt-free albumin. Clinical jaundice became apparent and bile appeared in the urine. He had vomited bloody fluid on two occasions shortly before he died.

Laboratory studies are summarized in Table V.

*Autopsy:* The significant findings were severe portal cirrhosis with ruptured esophageal varices and hemorrhage into the gastro-intestinal tract. A chronic congestive splenomegaly and abdominal ascites of approximately 1500 ml. of bile-stained fluid were present. There was great dilatation of the esophageal, gastric, paraumbilical and hemorrhoidal veins. The parietal peritoneal veins were distinctly dilated, particularly of both lateral peritoneal gutters and in the left upper abdomen where the greater omentum was adherent. The dilated omental veins originating from the stomach communicated with the lateral parietal veins in the left upper abdomen (Figures 9a, b and 10a, b). Most of this latter plexus of veins formed a single vein, which ran ventrally and emerged superficially about 2 inches above the umbilicus. There it received a small tributary from the dilated plexus of veins in the right peritoneal gutter which in turn communicated with intestinal veins via the mesentary. After becoming superficial the abdominal vein coursed cephalad and pierced the deep tissues in the region of the xiphisternum slightly to the right. It then turned to the left and joined the left internal mammary vein on the posterior surface of the anterior chest wall. This in turn entered the left innominate vein (Figures 9 and 10).

*N. N.*, a 51 year old German truck driver, was admitted to the Medical Service of the University Hospitals 27 June and discharged 23 August, 1949. There had been a brief visit to the Medical Out-Patient Clinic in October, 1938, when an anxiety state was suspected. At that time he was drinking beer occasionally. There was no evidence of liver disease. He reported a severe bout of malaria with coma two years previously.

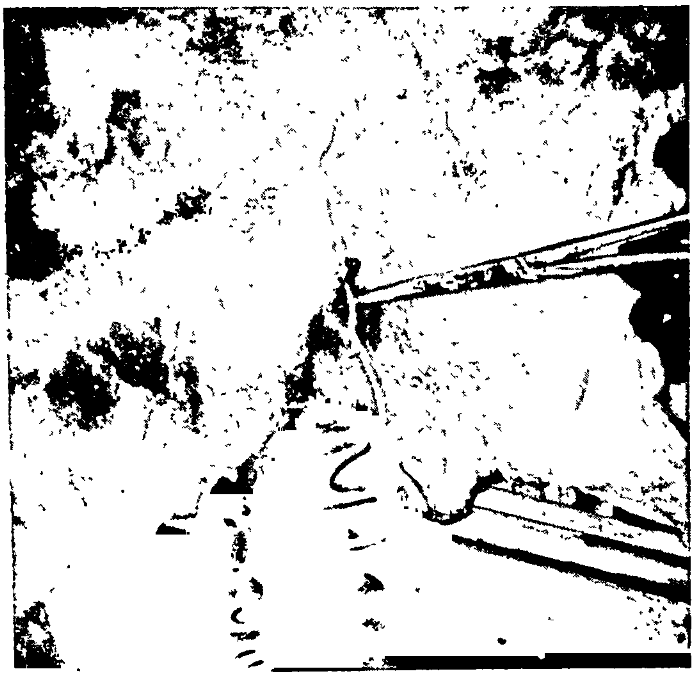

Fig. 10a. Photograph Showing Anastomotic Vein Piercing Anterior Surface of Chest Wall in Region of Xiphisternum

Fig. 10b. Photograph of Posterior Surface of Anterior Chest Wall Showing Left Internal Mammary Vein Which Has Just Been Joined by the Anastomotic Vein Described in Figure 10a.

During the year before admission insidiously beginning fatigue had progressed to a state of incapacity for the last three months. One month before entry abdominal discomfort, bloating and anorexia dominated the scene. There was diarrhea. Chilly feelings and fever occurred repeatedly until a week before admission when he took quinine. The urine became dark three weeks before admission but he had not noticed jaundice or itching. His weight declined from 170 to 135 pounds in spite of increase in his girth which interfered with comfortable breathing.

Examination revealed an ill man, whose swollen belly contributed to orthopnea. The skin was not jaundiced but exposed areas were tanned. Vascular spiders were scattered over the chest, arms and shoulders. The sclerae were icteric. Retinal vessels showed moderate sclerosis. The left side of the chest was restricted in movement and the trachea deviated to the right. Signs of fluid were noted on the left. A harsh systolic murmur, transmitted into the neck, was heard in the second and third interspaces on the right; and $P_2$ was accentuated. Fluid distended the abdomen. The firm irregular edge of the liver was felt 5 cm. below the rib margin on the right. The spleen was not felt. Pitting edema of the ankles was noted. Body and axillary hair were plentiful. A large anastomotic vein arising midway between umbilicus and xiphoid carried blood upwards. Large hemorrhoids were found.

Laboratory studies are summarized in Table V.

Treatment consisted of bedrest, high-protein, high-carbohydrate and low-salt diet supplemented with vitamins. Crude liver extract and mercuhydrin were given at frequent intervals. The left side of the chest was tapped on two occasions and 800 ml. of fluid removed each time. A number of abdominal paracenteses were performed with removal of 1–2 liters. Absorption and pressure studies were done on 15 August, 1949. The jaundice diminished and there was some clinical improvement, but he was still accumulating fluid at the time of discharge. He died at home on 1 September, 1949.

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# EFFECT OF CORTISONE ON ACUTE STREPTOCOCCAL INFECTIONS AND POST-STREPTOCOCCAL COMPLICATIONS[1]

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(Submitted for publication November 22, 1950; accepted, December 11, 1950)

It has been established that adrenal hormones play an important role in the defense reaction of the body to stress (1). A common stress stimulus in man is that produced by infection with group A streptococci. Streptococcal infections assume especial importance because they precede acute rheumatic fever, a disease which responds favorably to treatment with the adrenal hormone, cortisone (2–4). An epidemic of streptococcal exudative tonsillitis and pharyngitis afforded the opportunity to obtain data on the effect of cortisone therapy not only on an acute bacterial infection and the immunological response of the host, but also on the subsequent occurrence of acute rheumatic fever.

## DESCRIPTION OF STUDY

This study was conducted at Francis E. Warren Air Force Base, Wyoming, from February 20 to April 17, 1950. All patients entering the hospital with respiratory infections were examined within a few hours after admission. Since it was desirable to include in the study only those patients in the early phase of a streptococcal respiratory infection who would be available for follow-up examinations, the following criteria were employed: (*a*) the presence of exudate on the pharynx or tonsils, (*b*) a total leucocyte of 10,000 or greater, (*c*) an illness which began less than 31 hours before admission to the study, and (*d*) availability for follow-up studies for at least four weeks.

One hundred and seventy-four patients fulfilled the above criteria and were divided into treated and control groups on the basis of their Air Force serial number. Eighty-seven patients had numbers ending in the digits 5–9 and received treatment; 87 with numbers ending in the digits 0–4 received no specific therapy and composed the control group.

Cortisone acetate was injected intramuscularly into the gluteal region according to one of the two treatment schedules. The first 17 patients received a total of 500 mgms in 50 mgm doses. The first injection was given at the time of admission to the study and subsequent doses were administered at 12 hour intervals. The remaining 70 cases were given a total of 600 mgms in 100 mgm doses. The initial injection was given at the time of admission to the study, the second and third at 9 a.m. and 9 p.m. on the second hospital day, and the remaining three at 9 p.m. of the third, fourth and fifth days. Control patients received 0.85 per cent sodium chloride injections by the same schedules. During hospitalization no antipyretics or other medications were given except codeine for relief of severe headache.

Patients were examined daily through the sixth day of illness by one member of the professional staff who had no knowledge of the treatment being administered. Symptoms were recorded for each 12 hour period during the first two days of illness and daily thereafter. Physical signs were recorded daily. Oral temperatures were taken every four hours. Total leucocyte counts and cultures of the tonsils and pharynx were obtained each day. Sera were obtained on the first and sixth days of illness. An electrocardiogram was recorded soon after the acute symptoms had subsided.

Subsequent examinations were conducted during the third, fourth and fifth weeks following the onset of the streptococcal illness. The patients were questioned concerning post-streptococcal complications. Those with symptoms were examined and admitted to the hospital for further study. An electrocardiogram was taken approximately 21 days after onset. In addition, at each of the weekly examinations, serum and a culture of the throat were obtained.

All cultures were examined for *beta*-hemolytic streptococci which, when present, were isolated in pure culture. The first and last strains isolated during hospitalization and all strains obtained at each subsequent examination were grouped and typed according to the methods of Maxted (5, 6) and Swift, Wilson, and Lancefield (7). The acute and convalescent antistreptolysin "O" titers of all sera from each patient were determined concomitantly by a modification of the technique of Hodge and Swift (8).

## RESULTS

That the patients in this study had streptococcal disease is indicated by the demonstration of a significant rise[3] in the antistreptolysin titer in the sera of 86 per cent of the control group. In addition, group A streptococci were isolated from the initial cultures of the tonsils and pharynx of 89 per cent of all patients and the symptoms and physical signs were characteristic of streptococcal infections (9). The data presented in Table I show that the two groups of patients were essentially comparable.

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[1] This investigation was supported through the Commission on Acute Respiratory Diseases, Armed Forces Epidemiological Board, Office of The Surgeon General, Washington, D. C.

[2] Capt. MC, AUS.

The effect of cortisone on selected symptoms and physical signs is presented in Figures 1 and 2.[4] Little or no effect on the acute illness was demonstrated. The symptom, sore throat, persisted somewhat longer in the control group, being present on the sixth day of observation in 20 per cent of the control patients and 6 per cent of the treated cases. Chilliness, headache and malaise occurred with equal frequency in the two groups. No difference was noted between the treated and control patients in the time of disappearance of physical signs.

TABLE I

*Comparability of the 87 cases in each of the treated and control groups*

| | Cortisone group *per cent* | Control group *per cent* |
|---|---|---|
| History of tonsillectomy | 26.4 | 31.0 |
| Age 17–21 | 77.0 | 74.7 |
| *Symptoms:** | | |
| Chilliness | 65.5 | 75.8 |
| Feverishness | 82.7 | 86.2 |
| Malaise | 41.3 | 56.3 |
| Sore Throat | 82.7 | 85.0 |
| Headache | 86.2 | 80.4 |
| *Physical signs:†* | | |
| Enlarged or tender cervical nodes | 91.8 | 90.8 |
| Lymphoid hyperplasia | 37.9 | 37.9 |
| *Laboratory:* | | |
| Group A streptococci on admission | 89.7 | 88.5 |
| Type 14 | 54.0 | 65.5 |
| Type 24 | 17.2 | 10.3 |
| Type 6 | 9.1 | 9.1 |
| Leucocyte count 12,000 or greater | 91.9 | 86.2 |
| Antistreptolysin titer—125 units or less on admission | 68.9 | 67.8 |

* Symptoms occurring during first 12 hours of illness.
† Physical signs present on admission examination.

The number of patients with fever is recorded in Figure 3. At each four hour time period prior to treatment, the incidence of fever was the same in the two groups. Following therapy, the per cent of patients with fever was greater in those who received cortisone than in the control group. This difference, evident up to the third day of treatment, was as great as 26 per cent.

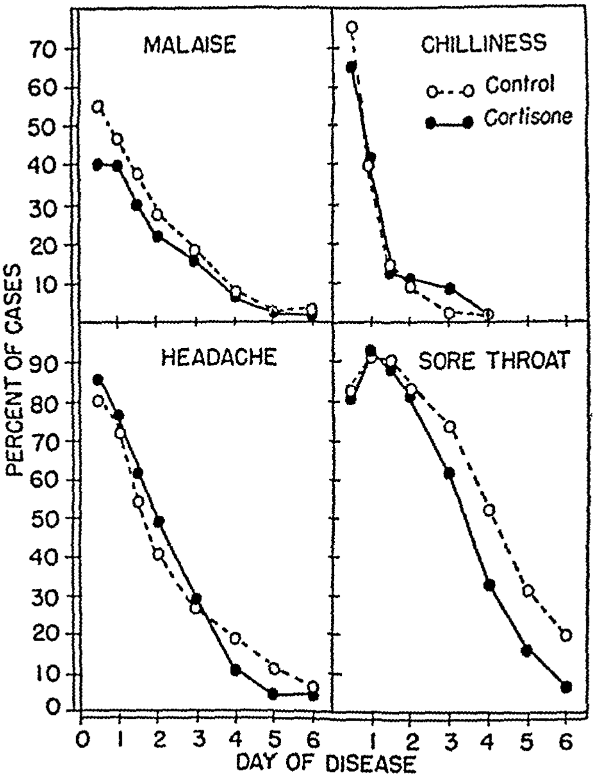

FIG. 1. EFFECT OF CORTISONE THERAPY ON THE SYMPTOMS OF STREPTOCOCCAL TONSILLITIS AND PHARYNGITIS

There were 15 patients who developed a complication during the period of treatment with cortisone or saline injections (Table II). Two of the

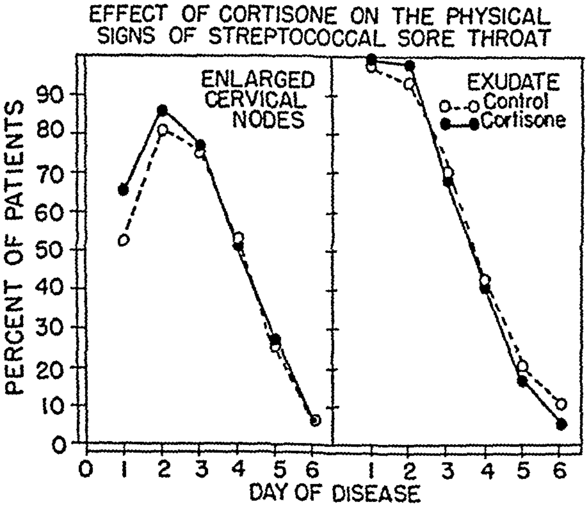

FIG. 2

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[3] A significant rise is considered to be two dilution increments when the convalescent titer is compared to the acute titer.

[4] Observations made after the development of purulent complications in four patients from the control group were not included in this analysis.

TABLE II

*The effect of cortisone on the complications of streptococcal sore throat*

| Complication | Cortisone group (87 cases) | Control group (87 cases) |
|---|---|---|
| Suppurative: | | |
| A. During treatment | | |
| 1. Peritonsillar cellulitis | 0 | 3 |
| 2. Otitis media | 4 | 7 |
| 3. Appendicitis | 1 | 0 |
| B. Following treatment | | |
| 1. Exudative tonsillitis | 4 | 3 |
| 2. Peritonsillar cellulitis | 1 | 2 |
| 3. Otitis media | 1 | 0 |
| Non-suppurative: | | |
| Rheumatic fever | 2 | 5 |

three peritonsillar abscesses required incision and drainage, and in one instance of acute otitis media spontaneous rupture occurred. All three of these patients from the control group received penicillin as therapy. Symptoms of otitis media were present in six untreated and four treated patients but were not severe enough to require specific therapy. In the group treated with cortisone, one patient developed acute appendicitis, the symptoms of which began 20 hours after the initial injection. With continued administration of cortisone, abdominal tenderness and rigidity, increase in pulse rate, and an elevated leucocyte count persisted. At operation, 20 hours after the onset of abdominal symptoms, a gangrenous appendix was removed. No *beta*-hemolytic streptococci were isolated from cultures of the appendix taken at the time of operation.

Suppurative complications, which became evident within ten days after the period of hospital observation, occurred in six patients in the treated group and in five in the control group. In the group receiving cortisone four patients with recurrences of exudative tonsillitis, one with peritonsillar cellulitis and one with acute otitis media were readmitted to the hospital. In the control group, there were readmissions for exudative tonsillitis in three cases and peritonsillar cellulitis in two patients.

A total of seven patients developed a non-suppurative sequela diagnosed as definite rheumatic fever according to a modification (10) of the classification of Jones (11). Two of the patients with rheumatic fever had received cortisone and five had received no therapy. All seven patients exhibited migrating polyarthritis, fever, and an elevated sedimentation rate. A past history of rheumatic fever was obtained in only one patient who was from the control group. The electrocardiographic abnormalities are recorded in Table III. The interval from the onset of the observed respiratory infection to the first symptom of rheumatic fever was 18 and 27 days in the treated group and 13, 19, 19, 27 and 28 days in the control patients. None of the seven patients showed bacteriological evidence of an intervening streptococcal infection, although one patient from the control group (interval 13 days) entered the hospital with peritonsillar cellulitis four days before the first symptom of rheumatic fever.

There were 15 patients who exhibited abnormalities of the P-R interval on the electrocardiogram recorded about 21 days after the onset of exudative tonsillitis or pharyngitis (Table III). Of the patients who received cortisone the P-R interval was greater than 0.21 seconds in five, and only one of these had symptoms of rheumatic fever. In the control group of patients four showed a prolongation of the P-R interval, three of whom had symptoms of rheumatic fever. In addition, there were three patients in the treated and three patients in the control group whose P-R interval increased at least 0.04 second on the 21st day as compared to the initial recording.

Of the group of eight patients who showed abnormal P-R intervals on the electrocardiogram and who showed no other evidence of rheumatic fever, four in the cortisone treated group showed bacteriological evidence of a new infection. A new type of streptococcus was isolated from the phar-

TABLE III

*Effect of cortisone therapy of acute streptococcal infections on the post-streptococcal electrocardiogram*

| Classification | Cortisone group | | Control group | |
|---|---|---|---|---|
| | Total cases | Cases | Total cases | Cases |
| Rheumatic fever: | 2 | | 5 | |
| P-R interval greater than 0.21 second | | 1 | | 3 |
| Increase P-R interval of at least 0.04 second* | | 1 | | 2 |
| No symptoms of rheumatic fever: | 85 | | 82 | |
| P-R interval greater than 0.21 second | | 4 | | 1 |
| Increase in P-R of at least 0.04 second* | | 2 | | 1 |
| Totals | 87 | 8 | 87 | 7 |

* Exclusive of individuals showing a P-R interval of over 0.21 second.

ynx of three patients at the time of the second electrocardiogram but there was no other evidence of infection. In one patient a new type of streptococcus was isolated at the time of readmission to the hospital for exudative tonsillitis seven days after the onset of the original infection.

Total leucocyte counts were obtained daily during hospitalization in 96.6 per cent of the cases. The percentage of leucocyte counts greater than 10,000 is recorded in Table IV according to hospital day. The number of elevated leucocyte counts fell continuously in the control group. In contrast, the treated group showed a decrease in elevated counts on the second day followed by a return to a high level and then a gradual fall.

Group A streptococci were isolated at some time during hospitalization in 95.3 per cent of the cortisone treated group and 96.5 per cent of the control group. The same type of organism was isolated during the acute illness and throughout the follow-up period in 79.5 per cent of the treated group and 71.6 per cent of the untreated group. A new type of streptococcus was identified in 8.5 per cent of the cortisone group and 4.9 per cent of the control group. All of these changes in type, except for one instance in the control group, were demonstrated after discharge from the hospital.

TABLE IV

*Effect of cortisone therapy in streptococcal sore throat on the total leucocyte count*

| Group | Per cent of leucocyte counts greater than 10,000 according to hospital day | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| Cortisone | 95.1 | 78.7 | 90.7 | 83.5 | 78.8 |
| Control | 92.9 | 83.5 | 54.7 | 48.2 | 48.1 |

The antistreptolysin titers of the sera collected on admission to the hospital and five to seven days after the onset of the disease are recorded in Figure 4. Since the distribution of the antistreptolysin titers of the acute phase sera is very similar in the two the average rise in the antibody titer during convalescence may be compared.

At the end of the first week of illness the sera of the control group showed an average rise of 0.770 dilutions (Table V) as compared to a rise of 0.541 in the cortisone treated patients. During *the subsequent three weeks the average increase* in antibody was somewhat greater in the treated group than in the patients who received no therapy. These differences were 0.175, 0.181, and 0.332 dilution increments at the second, third and fourth weeks, respectively.[5]

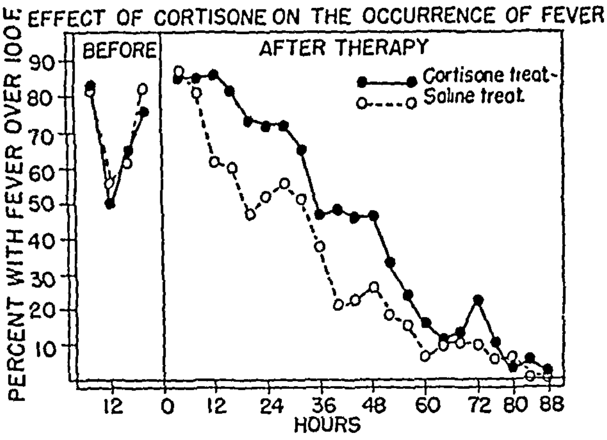

FIG. 3

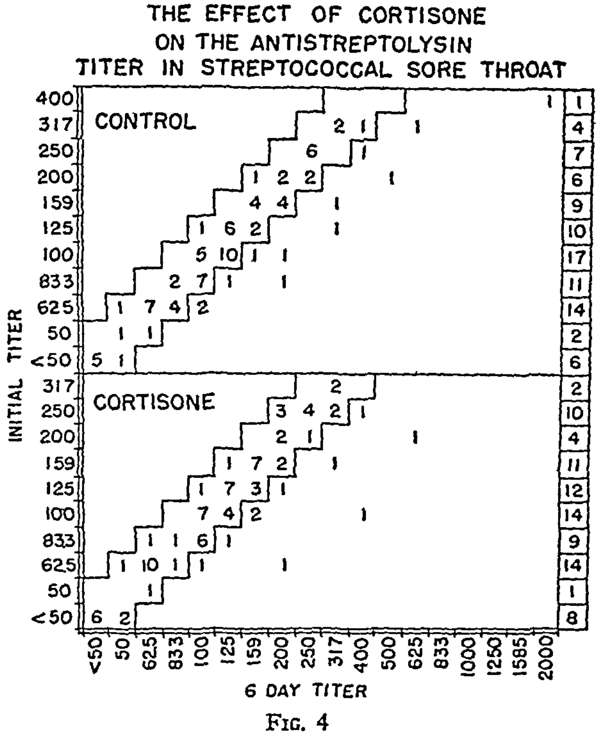

FIG. 4

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[5] In the treated series, those patients who gave a history of tonsillectomy had a higher antistreptolysin response than those with no such history. The average dilution increment in those without tonsils at the end of two, three and four weeks was 4.454, 5.173, and 5.173, respectively, as compared to 3.350, 3.650, and 3.542 in the patients with tonsils. No difference was noted in the control group.

TABLE V

*The effect of cortisone on the antistreptolysin titer* *

| Group | Days after onset of illness | Number of sera | Average dilution increase in antistreptolysin titer † |
|---|---|---|---|
| Cortisone | 5–7 | 84 | 0.541 |
| Control | | 84 | 0.770 |
| Cortisone | 14–18 | 79 | 3.607 |
| Control | | 81 | 3.432 |
| Cortisone | 20–25 | 78 | 4.012 |
| Control | | 83 | 3.831 |
| Cortisone | 27–32 | 75 | 3.973 |
| Control | | 78 | 3.641 |

* The three patients who received penicillin therapy are excluded.

† The initial dilutions of sera employed in the tests are recorded in Figure 4. Each change in titer of one dilution is assigned a value of one (plus or minus). All changes in titer are related back to the titer of the serum specimen obtained during the first 31 hours of illness.

The only untoward effect noted during cortisone therapy was soreness around the area of injection in all patients. The soreness was most marked during the first 48 hours of therapy and diminished somewhat by the time of the last injection.

## DISCUSSION

Adrenocorticotrophic hormone (ACTH) and adrenal cortical extracts have been used in studies designed to determine the effect of these hormones on acute bacterial infections. Perla and Marmorston (12) treated 17 cases of bronchopneumonia, one case of induced malaria, and six severe "grippal" infections with adrenal cortical extract. These authors report that such therapy was beneficial in that it shortened the convalescent period. Jacobs (13) treated non-immunized children who had whooping cough with adrenal cortical extract and reported the mildest course in this group when compared to other similar groups receiving pertussis antigens or triple typhoid vaccine. More recently, Finland, Kass, and Ingbar (14) used ACTH in the treatment of one case of pneumococcal pneumonia and one patient with virus pneumonia. These patients showed rapid symptomatic improvement.

In contrast to the above studies, the data presented here show that the adrenal hormone, cortisone, in the doses used, had no effect on the symptoms or physical signs of acute streptococcal sore throat. Furthermore, fever appeared to be prolonged in those patients receiving cortisone.

Whether cortisone therapy affects the development of complications can not be determined from the data obtained. There were ten such complications which developed in the control group during the period of treatment with saline and five in the cortisone treated group. There was one patient treated with cortisone who exhibited abdominal tenderness and rigidity, rapid pulse rate, fever and leucocytosis. This is in contrast to the report of Beck, Browne, Johnson, Kennedy and Mackenzie (15) who observed two patients who developed peritonitis during treatment with ACTH but who failed to exhibit the classical features of this disease.

Leucocytosis during the course of cortisone therapy as demonstrated in this study has been noted previously (16–19). The decrease in percentage of treated patients having leucocyte counts greater than 10,000 on the second day (Table IV) can possibly be explained by the fact that on this day most of the determinations were made about one hour after treatment. This drop in total leucocyte count immediately following the injection of ACTH and adrenal cortical extracts has been described (1, 20, 21). The remaining counts were taken about 13 hours following the previous injection.

A number of investigators have studied the relationship between adrenal hormones (22–30) and antibody production. Chase, White, and Dougherty (22) report that the administration of adrenal cortical extracts to animals receiving injections of staphylococcal toxin, horse serum, egg albumin or sheep cells results in higher antibody titers than obtained in control animals which received no adrenal extract. Mirick (23), however, reports that in man the administration of ACTH or cortisone does not alter the antibody response to immunization with pneumococcal polysaccharides. Finland, Kass, and Ingbar (14) observed that in one patient with pneumococcal pneumonia treated with ACTH antibodies appeared in the serum at about the expected time.

In contrast to the above reports, Fischel (24) demonstrated an inhibition of antibody formation in rabbits given ACTH during immunization with formalinized pneumococci. Gernfuth and Ottinger (25) report that ACTH and cortisone administration markedly suppressed the formation of antibody to egg albumin in rabbits. In these experiments 10 mgms of cortisone were administered daily for 18 days to rabbits weighing 2 kgms. Such therapy completely inhibited antibody formation to egg albumin. It is to be noted, however, that antibody was not measured several weeks after the experimental period was completed. The relative doses of cortisone employed in these rabbits were greater than those used in the present study.

Antibodies to various products of group A streptococci are especially high in patients with rheumatic fever (31, 32). Recently it has been demonstrated that the attack rate of rheumatic fever following streptococcal pharyngitis is related, in part, to the magnitude of the antibody response (33). Procedures which alter this antibody response may also alter the incidence of subsequent rheumatic fever. Thus penicillin therapy of acute streptococcal pharyngitis inhibits the formation of antibody presumably because of the eradication of the antigen (streptococci) from the throat and at the same time such treatment markedly decreases the incidence of acute rheumatic fever (10).

Cortisone therapy as employed in the present study failed to alter appreciably the antistreptolysin response to streptococcal infections. Because the sera of the treated group showed a somewhat lower antibody titer at six days than the sera of the control group there may have been a slight inhibition of antibody formation. It should be emphasized, however, that the increase in antibody was somewhat greater in the treated group than in the control patients two, three and four weeks after the onset of illness. This may be due to the fact that cortisone is not antibacterial, so that when treatment was discontinued the antigenic stimulus was still effective.

The effect of cortisone therapy on acute streptococcal infections is of especial interest not only because of the above considerations, but also because the compound also exerts a remarkable effect when administered to patients with rheumatic fever (2–4). Acute rheumatic fever was not prevented by cortisone therapy. Although there were only two cases in the treated group and five cases among the control patients, this distribution could have occurred by chance. Since the incidence of abnormal electrocardiograms was equal in the treated and control group of patients, cortisone therapy had no effect on these signs which are frequently associated with rheumatic fever. Since there may have been a slight inhibition of antibody formation during the period of therapy, it is entirely possible that larger doses of cortisone administered for a longer period of time may inhibit antibody production and reduce the incidence of rheumatic fever.

## SUMMARY

Eighty-seven treated and 87 control patients were studied to determine the effect of cortisone on the clinical and immunological response to streptococcal respiratory infections. Cortisone therapy, in the dosages employed, exerted no effect on the symptoms or physical signs of this infection. Patients who received cortisone exhibited fever for a longer period of time than the control patients. Suppurative complications developed during the period of cortisone or saline injections in one of the treated patients and in three of the control subjects.

Rheumatic fever was diagnosed in two treated and five control subjects. The P-R interval recorded approximately three weeks after the onset of illness was greater than 0.21 second in a total of five of the treated and four of the control group.

The increase in the antistreptolysin titer was somewhat less in the sera of the treated group than in the control patients one week after the onset of the infection, but at two, three and four weeks the formation of antibody was slightly greater in treated than in the control patients.

## ACKNOWLEDGMENTS

To Col. John K. Cullen and Col. Howard F. Currie, Post Surgeons, and Capt. James G. Hirsch, chief of Medical Service, we express our deep appreciation, for without their cooperation this study would have been impossible.

We are pleased to acknowledge the assistance of the laboratory staff: Mary E. Riner, technician in chief, Donna Carpenter, Joanne Merrill, Emily K. Lambert, Phyllis Guthrie, Elaine Jessen, John Datillo, T/Sgt.

Earl C. Marple, Sgt. Joe Page, Sgt. Frederick N. Barkman, and Pfc. Ray F. Long.

We also wish to thank the following ward personnel for their admirable performance of ward duties far in excess of the usual load: Sgt. Charles W. Sass, Cpl. Norris E. Dieble, Cpl. Melvin L. Wood, Cpl. John W. Roberts, and Pfc. Alberto J. Romero.

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# THE MECHANISM OF POLYURIA OF DIABETES INSIPIDUS IN MAN. THE EFFECT OF OSMOTIC LOADING

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(Submitted for publication July 1, 1950; accepted, December 21, 1950)

## INTRODUCTION

Although there is general agreement on the role of the hypothalamic-hypophyseal region in the genesis of diabetes insipidus (1), the nature of the renal defect which results in the excretion of a copious and dilute urine has remained obscure. The excretion of a hypotonic urine in diabetes insipidus may be explained by any of three hypotheses: (*a*) the delivery of a hypotonic fluid from the proximal tubule, or (*b*) an isotonic proximal urine with solute reabsorption in the distal tubule, and (*c*) an isotonic proximal urine with water secretion in the distal tubule.

A complete decision with regard to these hypotheses is not at hand. Hypothesis (*a*), assuming a hypotonic proximal fluid (2), although presumably based on the observations of Walker and his associates (3) would require a much greater hypotonicity than ever observed in order to explain the low urinary osmolarity of diabetes insipidus. Hypothesis (*b*) (4), most widely accepted at present, holds that the polyuria is due to lack of water reabsorption in the distal renal tubule. Normally ⅞ of the glomerular filtrate is reabsorbed isotonically in the proximal tubule, while ⅛, under the hormonal control of the hypothalamic-hypophyseal region, may be reabsorbed in the distal tubule. Lack of hormone results in a maximum diuresis of about ⅛ of the glomerular filtration rate. This hypothesis, in order to explain the dilute urine of diabetes insipidus, requires the assumption of solute reabsorption in the distal tubule, with a distal reabsorbate of unspecified but hypertonic osmolarity. Hypothesis (*c*), popular before the general acceptance of the glomerular filtration theory, has for its major objection the demonstrated proportionality between urine flow and arterial pressure in the isolated kidney (5).

It appeared that osmotic diuresis in patients with diabetes insipidus might offer a means of testing the three hypotheses. Given an unchanged glomerular filtration rate, and assuming with Shannon (2) that the urine formed in diabetes insipidus resembles that which is delivered to the distal tubule, no significant change in urinary osmolarity should occur after loading. The predictions of hypothesis (*b*) depend on the nature of the distal reabsorptive process assumed. Hypothesis (*c*) would require that, given a constant rate of secretion of hypotonic urine prior to loading, the increment of urine flow during osmotic diuresis, representing additional proximal fluid, be nearly isotonic with plasma.

Osmotic diuresis with mannitol loading was produced in three subjects with diabetes insipidus. The rates of urine flow and solute excretion before and during loading as well as those of glomerular filtration and renal plasma flow were studied.

## EXPERIMENTAL

The subjects of the present study were one woman, 19 years, and two boys, six and 12 years of age, with diabetes insipidus. They had abstained from food and drink for four to eight hours, and from pitressin medication for two to three days preceding the test.

Mannitol, in 25% solution, was administered as the loading solute. Two priming injections, the first of 180 and the second of 90 milliosmoles (mOsm.) per 1.73 $M^2$, calculated to add 20 and 10 mOsm., respectively, per liter of distribution volume, were given, followed by a maintenance infusion in the amount of 1% of the priming dose per minute. All infusion fluids had sufficient para-aminohippurate (PAH) added to maintain a plasma level of about 3 mgm./100 cc., for the measurement of renal plasma flow. After a 20 minute period of equilibration following the priming infusion, urine was collected for two or three periods of 15–20 minutes each at both loading levels and blood samples were drawn at appropriate intervals. The urine and blood were analysed for mannitol, urea, sodium, potassium, chloride, and PAH. Osmotic activity was determined directly by the freezing point in urine, and calculated from the determined[2] solutes in blood. The analytical techniques used have been described (6).

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Toward the end of the test periods, the patients became weak and listless and the skin felt cold to touch. One patient, D. S., complained of headache, and another vomited repeatedly when he drank water after the test.

The tests were repeated on two of the patients, D. E. and I. W., after an interval of three to four weeks, while they were receiving pitressin. Five-tenths cc. of an aqueous solution of pitressin (10 pressor units) was given intramuscularly every three hours for 20 hours preceding, and every two hours during the test. On pitressin, the patients were able to withstand 16 hours of water deprivation, and experienced no untoward symptoms during the loading procedure.

## RESULTS

Table I presents results on three patients obtained in five tests comprising 40 periods of urine collection. Data include the corrected minute volume of urine, the osmolarity of urine and plasma, clearance of mannitol and PAH, and the filtration fraction.

It may be seen that during the pre-loading periods, the urine flow remained at high values of 5.1–11 cc./min., despite the hydropenic state of the subjects. The urine was hypotonic, its osmolarity reaching values as low as 56 mOsm./L., or approximately ⅙ that of plasma. After loading, with increasing flows, the osmolarity of the urine increased. The highest value obtained, 232 mOsm./L., was slightly more than ⅔ of the plasma

TABLE I

*Urine flow, osmolarity, and clearances of mannitol and PAH during mannitol diuresis*

*I. W., 2-25-50, S.A. 1.45 M², Mannitol, No pitressin:*

| Period no. | Concurrent time | Urine volume | Osmolarity urine | Osmolarity plasma | Clearance mannitol | Clearance PAH | Clearance mann. / Clearance PAH |
|---|---|---|---|---|---|---|---|
| | *min.* | *cc./min./1.73 M²* | *mOsm./L.* | *mOsm./L.* | *cc./min./1.73 M²* | *cc./min./1.73 M²* | |
| P-1 | −76 to −46 | 5.14 | 67 | | | | |
| P-2 | −46 to −18 | 6.76 | 58 | | | | |
| P-3 | −18 to −6 | 7.31 | 56 | 285 | | | |
| | 0 to 7.0 | | 121 cc. of 25% Mannitol | | | | |
| X-I | −6 to 20 | 14.0 | 124 | | | | |
| 1 | 20 to 30 | 15.5 | 158 | 291 | 104.2 | 498 | 0.21 |
| 2 | 30 to 40 | 14.4 | 155 | 295 | 92.2 | 451 | 0.20 |
| | 42 to 50 | | 104 cc.of 25% Mannitol | | | | |
| X-II | 40 to 62 | 19.6 | 185 | | | | |
| 3 | 62 to 80 | 17.6 | 208 | 300 | 90.8 | 496 | 0.18 |
| 4 | 80 to 90 | 14.9 | 228 | 304 | 84.9 | 472 | 0.18 |
| 5 | 90 to 102 | 14.5 | 232 | | | | |
| *D. E., 12-8-49, S. A. 0.81 M², Mannitol, No pitressin:* | | | | | | | |
| P-1 | −71 to −32 | 6.92 | 132 | | | | |
| P-2 | −32 to −10 | 7.58 | 113 | 300 | | | |
| | 0 to 10 | | 69 cc. of 25% Mannitol | | | | |
| X-I | −10 to 21 | 11.8 | 159 | | | | |
| 1 | 21 to 40 | 11.3 | 170 | 316 | 54.5 | 325 | 0.17 |
| 2 | 40 to 58 | 10.7 | 184 | 317 | 52.0 | 329 | 0.16 |
| | 60 to 69 | | 36 cc. of 25% Mannitol | | | | |
| X-II | 58 to 75 | 14.7 | 195 | | | | |
| 3 | 75 to 93 | 15.2 | 200 | 324 | 55.4 | 388 | 0.14 |
| 4 | 93 to 125 | 11.0 | 212 | 332 | 43.9 | 256 | 0.17 |

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[2] Osmotic Activity of Plasma $= [2(\mathrm{Na} + \mathrm{K}) - 8 + \mathrm{Urea} + \mathrm{Mannitol}] \times \gamma$, where $\gamma$, the activity coefficient of plasma, was assumed to be 0.92.

TABLE 1—*Continued*

*D. S., 2-6-50, S. A. 0.83 M², Mannitol, No pitressin:*

| Period no. | Concurrent time | Urine volume | Osmolarity urine | Osmolarity plasma | Clearance mannitol | Clearance PAH | Clearance mann. / Clearance PAH |
|---|---|---|---|---|---|---|---|
| | *min.* | *cc./min./1.73 M²* | *mOsm./L.* | *mOsm./L.* | *cc./min./1.73 M²* | *cc./min./1.73 M²* | |
| P-1* | −69 to −37 | 10.7 | 56 | | | | |
| | 0 to 10 | 71 cc. of 25% Mannitol I.V. | | | | | |
| X-I | −37 to 16 | 13.1 | 79 | | | | |
| 1 | 16 to 26 | 27.9 | 130 | | | | |
| 2 | 26 to 37 | 18.8 | 135 | | | | |
| | 39 to 49 | 37 cc. of 25% Mannitol | | | | | |
| X-II | 37 to 50 | 19.8 | 147 | | | | |
| 3 | 50 to 59 | 21.0 | 146 | | | | |

*D. E., 1-11-50, S.A. 0.82 M², Mannitol, Pitressin:*

| Period no. | Concurrent time | Urine volume | Osmolarity urine | Osmolarity plasma | Clearance mannitol | Clearance PAH | Clearance mann. / Clearance PAH |
|---|---|---|---|---|---|---|---|
| P-1 | −94 to −46 | 1.15 | 777 | | | | |
| P-2 | −46 to −12 | 1.52 | 747 | 263 | | | |
| | 0 to 7.4 | 61 cc. of 25% Mannitol | | | | | |
| X-I | −12 to 35 | 5.91 | 583 | | | | |
| 1 | 35 to 60 | 6.50 | 605 | 269 | 145.5 | 597 | 0.24 |
| 2 | 60 to 78 | 6.18 | 602 | 271 | 135.2 | 554 | 0.24 |
| | 79 to 84 | 40 cc. of 25% Mannitol | | | | | |
| X-II | 78 to 102 | 8.29 | 551 | | | | |
| 3 | 102 to 130 | 8.31 | 538 | 277 | 118.5 | 513 | 0.23 |
| 4 | 130 to 157 | 8.84 | 511 | 277 | 132.8 | 469 | 0.28 |

*I. W., 3-25-50, S.A. 1.45 M², Mannitol, Pitressin:*

| Period no. | Concurrent time | Urine volume | Osmolarity urine | Osmolarity plasma | Clearance mannitol | Clearance PAH | Clearance mann. / Clearance PAH |
|---|---|---|---|---|---|---|---|
| P-1 | −73 to −40 | 0.43 | 605 | | | | |
| P-2 | −40 to −3 | 0.76 | 677 | 303 | | | |
| | 0 to 11 | 121 cc. of 25% Mannitol | | | | | |
| X-I | −3 to 25 | 3.50 | 606 | | | | |
| 1 | 25 to 35 | 3.62 | 606 | 307 | 95.6 | 565 | 0.17 |
| 2 | 35 to 54 | 4.15 | 611 | 307 | 107 | 606 | 0.18 |
| | 58 to 69 | 104 cc. of 25% Mannitol | | | | | |
| X-II | 54 to 79 | 4.83 | 624 | | | | |
| 3 | 79 to 89 | 5.33 | 608 | 314 | 95.5 | 405 | 0.24 |
| 4 | 89 to 99 | 6.45 | 605 | 318 | 104 | 510 | 0.20 |

osmolarity. Urine flows increased two- to threefold over the preliminary rates to values of 15–28 cc./min. These effects are the reverse of those observed in normal hydropenic subjects during solute loading, where with increasing flows urinary osmolarity decreased but remained greater than that of plasma (6).

The last two tests shown in the table were performed on patients D. E. and I. W. while they were under the influence of pitressin. During the pre-loading periods, the urine flow varied between 0.43 to 1.15 cc./min.[3] After loading, with flows attaining values as high as 8.8 cc./min., urinary osmolarity decreased to a value of 511 mOsm./L. The results were essentially the same as those observed in normal hydropenic subjects after mannitol loading (6).

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[3] The high preliminary flows observed in patient D. E. perhaps may be explained by a mild electrolyturetic action of the pitressin.

The clearances of mannitol and PAH were low in patient D. E., a 12 year old dwarfed boy. When the test was repeated with the patient on pitressin, the clearance of mannitol increased three-fold and that of PAH, two-fold, with an increase in the filtration fraction. Decreases in filtration rate have been observed in dogs with diabetes insipidus (7). In the case of patient I. W., the clearances of both mannitol and PAH were normal and did not change significantly after pitressin administration.

It is evident that in each patient, the clearance values remained unchanged during the tests. Although no clearances were determined in the preliminary periods, one may assume that they changed little with loading both from the constancy of the clearances at different levels of loading and from data in the literature on normal subjects and dogs (8).

The independence of urine flow from changes of filtration rate or renal plasma flow is evident from the data shown in the table.

Figure 1 contains a plot of the values for urine flow versus solute load. The curve drawn is based on the data of nine normal hydropenic subjects during mannitol diuresis. It can be seen that for a given solute load, the patients with diabetes insipidus excreted urine at a rate two to three times greater than normal subjects. On the other hand, when treated with pitressin, their response was normal. A dependence of urinary volume on the "total milliosmolar excretion rate" in patients with diabetes insipidus has been noted (9). It is of interest that both patients on pitressin excreted solutes in an identical manner despite sizeable differences in their filtration rate.

Figure 2 shows plots of losses of sodium, chlo-

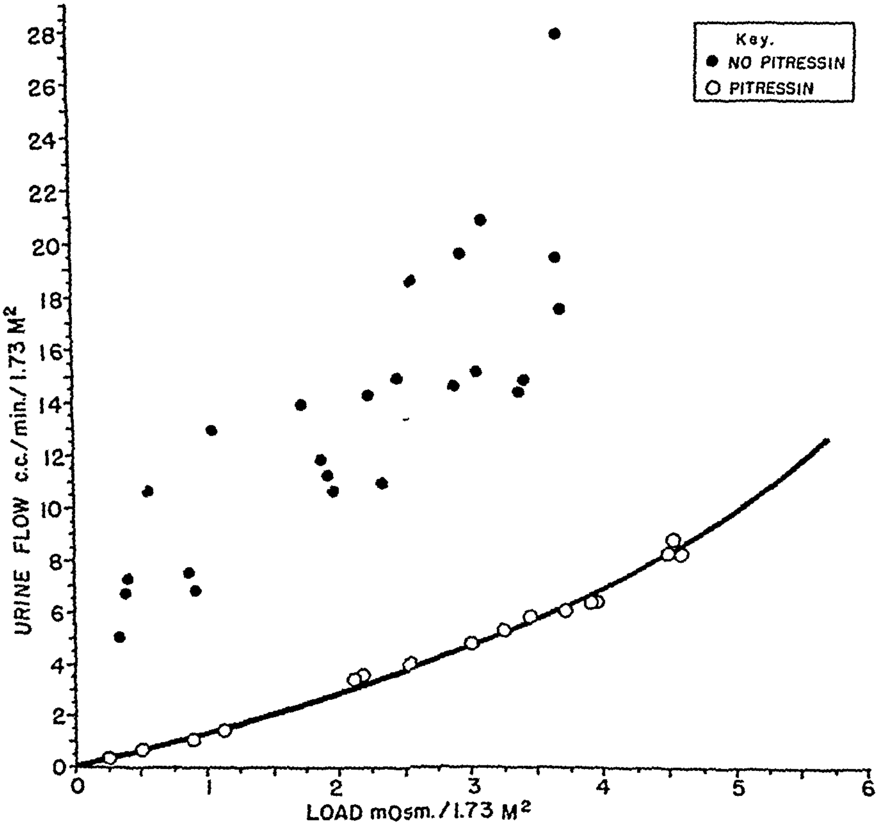

Fig. 1. Urine Flow versus Osmotic Load

Open and solid circles refer to the patients with diabetes insipidus, with and without pitressin therapy, respectively. The line represents the average flow-load curve of nine normal subjects.

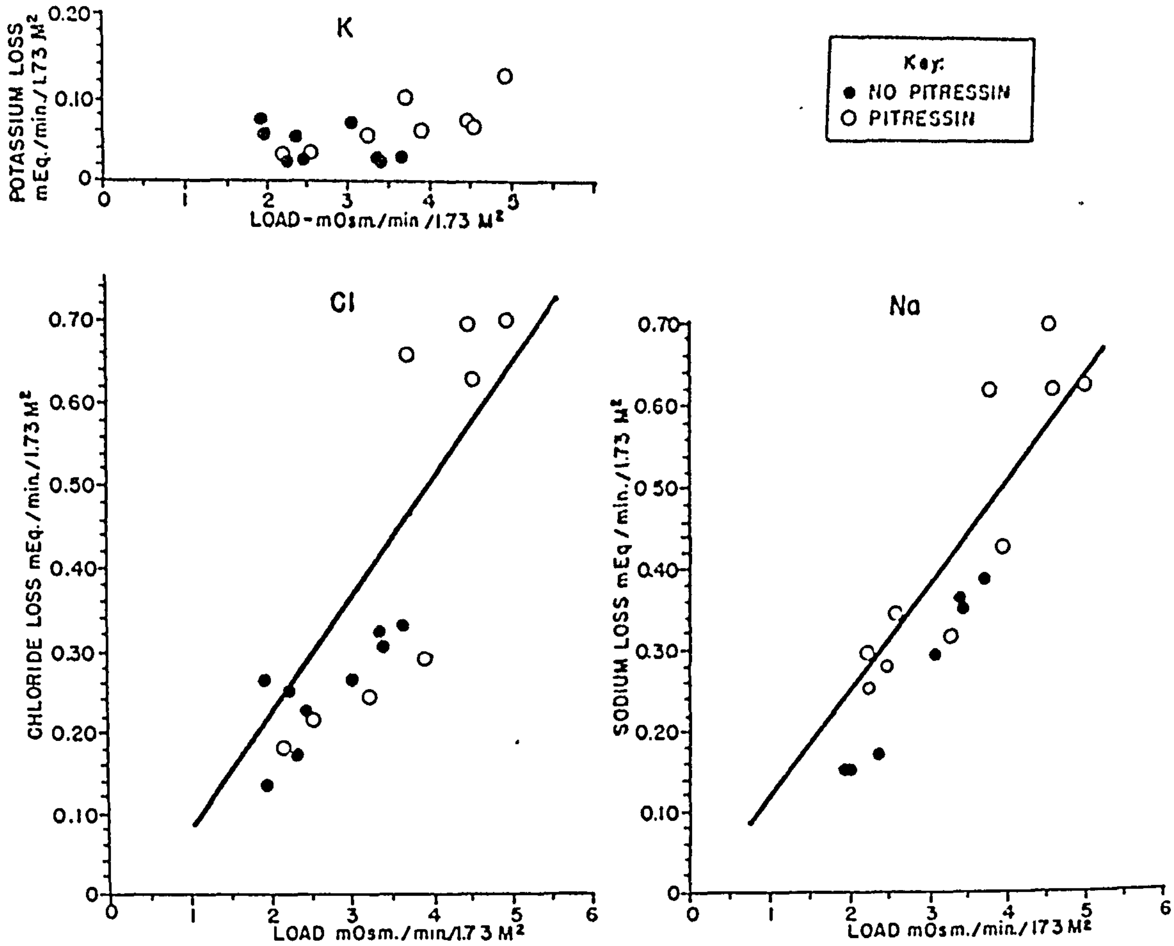

Fig. 2. Potassium, Chloride and Sodium Losses versus Osmotic Load during Mannitol Loading

The symbols have the same meaning as in Figure 1.

ride, and potassium versus total solute load. The lines represent linear regressions calculated from nine normal hydropenic subjects under conditions of osmotic loading with mannitol. It can be seen that the data group themselves about the lines in a fairly satisfactory manner. The pattern of salt loss was the same in both pitressin-treated and untreated patients, despite the wide disparity in urine flows. Solute loading had little effect on potassium excretion in the subjects of the present study as observed previously in normal subjects (10). Mannitol excretion, not plotted, accounted for the same percentage of urinary solutes (50–60%) as it did in normal subjects (6).

The linear relation of sodium, chloride, and mannitol to the total solute load indicates that the per cent composition of the urine in patients with diabetes insipidus during solute loading is similar to that of normal subjects. It also suggests that the final solute load destined for excretion is assembled in a portion of the tubule proximal to the site of concentration or dilution of the urine.

## DISCUSSION

The observed two- to four-fold increase of urinary osmolarity in patients with diabetes insipidus after osmotic loading appears incompatible with a hypothesis which requires the delivery of a hypotonic fluid from the proximal tubule. The converse hypothesis, namely, the flow of an isotonic proximal fluid, may be tested, given the additional assumption that there exists a basal, dilute urine flow, that observed in the preliminary periods, which changes little throughout the experiment. After loading, the increment of urinary volume and load above the control level should consist of isotonic proximal fluid. This may be calculated as the increment of urinary load divided by the plasma osmolarity. Table II contains the calculations of the urine flow based on these assumptions, compared with the observed values. The data represent 23 periods of urine collection, of which 18 were obtained during loading. It can be seen that the calculated and observed rates of flow were in

TABLE II

*Urine flow calculated on the assumption of constant basal flow and addition of isotonic proximal fluid*

| Patient | Period | (a) Load | (b) Assumed load added* | (c) Plasma osmotic activity | (d) Calculated values: Increment prox. fluid (b ÷ c) | (e) Calculated values: Urine† flow | (f) Observed flow urine |
|---|---|---|---|---|---|---|---|
| | | *mOsm./min.* | *mOsm./min.* | *mOsm./L.* | *cc./min.* | *cc./min.* | *cc./min.* |
| D. E. | P-1 | 0.92 | | 300 | | | 6.9 |
| | P-2 | 0.86 | | | | | 7.6 |
| | X-I | 1.88 | 0.99 | | 3.14 | 10.4 | 11.8 |
| | 1 | 1.93 | 1.04 | 316 | 3.29 | 10.5 | 11.3 |
| | 2 | 1.97 | 1.08 | 317 | 3.42 | 10.7 | 10.7 |
| | X-II | 2.87 | 1.98 | | 6.15 | 13.4 | 14.7 |
| | 3 | 3.04 | 2.15 | 329 | 6.67 | 13.9 | 15.2 |
| | 4 | 2.34 | 1.45 | 332 | 4.37 | 11.6 | 11.0 |
| I. W. | P-2 | 0.39 | | 285 | | | 6.8 |
| | P-3 | 0.41 | | | | | 7.3 |
| | X-I | 1.74 | 1.34 | | 4.57 | 11.6 | 14.0 |
| | 1 | 2.45 | 2.05 | 291 | 6.99 | 14.0 | 15.5 |
| | 2 | 2.23 | 1.83 | 295 | 6.24 | 13.3 | 14.4 |
| | X-II | 3.63 | 3.23 | | 10.7 | 17.7 | 19.6 |
| | 3 | 3.66 | 3.26 | 300 | 10.8 | 17.8 | 17.6 |
| | 4 | 3.40 | 3.00 | 304 | 9.93 | 17.0 | 14.9 |
| | 5 | 3.36 | 2.96 | | 9.80 | 16.8 | 14.5 |
| D. S. | P-1 | 0.60 | | | | | 10.7 |
| | X-I | 1.04 | 0.44 | | 1.56 | 12.3 | 13.1 |
| | 1 | 3.63 | 3.60 | 282 | 12.8 | 23.5 | 27.4 |
| | 2 | 2.54 | 1.94 | | 6.88 | 17.6 | 18.8 |
| | X-II | 2.92 | 2.32 | | 7.95 | 18.6 | 19.9 |
| | 3 | 3.07 | 2.47 | 292 | 8.46 | 19.2 | 21.0 |

Columns (a) and (b) present the values for the observed urinary load and its increment during loading; and column (c), the values for the osmotic activity of the plasma. The calculated increments of proximal fluid, *i.e.*, the increment of load divided by plasma osmolarity, are presented under column (d); calculated values for the urine flow, obtained by adding the calculated increment of isotonic fluid to the basal urine flow, under column (e); and the values for the observed urine flow, under column (f).

* The assumed load added is the difference between post-loading and average pre-loading solute loads.

† Sum of increment of proximal fluid (d) and basal flow, taken as the average of pre-loading flows.

reasonably close agreement, the maximum deviation being 17%. The close agreement between theoretical and observed urine flows supports the validity of both initial assumptions, *i.e.*, an unchanging basal flow and addition of isotonic fluid from the proximal tubule during loading. Since the method of calculation is independent of the nature of the basal urinary flow and load, the predictions hold for a hypothesis of solute reabsorption, if a constant rate of reabsorption[4] in the distal tubule both before and after loading is assumed, as well as for a hypothesis of distal secretion of water.

Direct proof for either hypothesis is not yet available. Much indirect evidence, martialled from phylogenetic, physiologic, and physico-chemical considerations, favors a process of a distal secretion rather than one of reabsorption.

That tubular secretion occurs in the aglomerular kidney of certain marine teleosts has been shown by Marshall (11), who found, in the absence of any filtration process, production of a urine isotonic with or hypotonic to the plasma. Bieter (12) added further evidence by showing that the secretion pressure in the ureter was greater than the blood pressure in the dorsal aorta. There is no *a priori* phylogenetic reason to deny the existence of a secretory function for water in higher animals with glomerular kidneys.

Data from studies on water diuresis in man or dog (13–16) may be interpreted in terms of tubular secretory activity. The ingestion of water is followed by a characteristic response in urine flow. After a brief lag period the urine flow increases to a maximum and declines sharply to the initial

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[4] The assumption of a variable rate of distal solute reabsorption would fail to explain the added flow or load observed during loading.

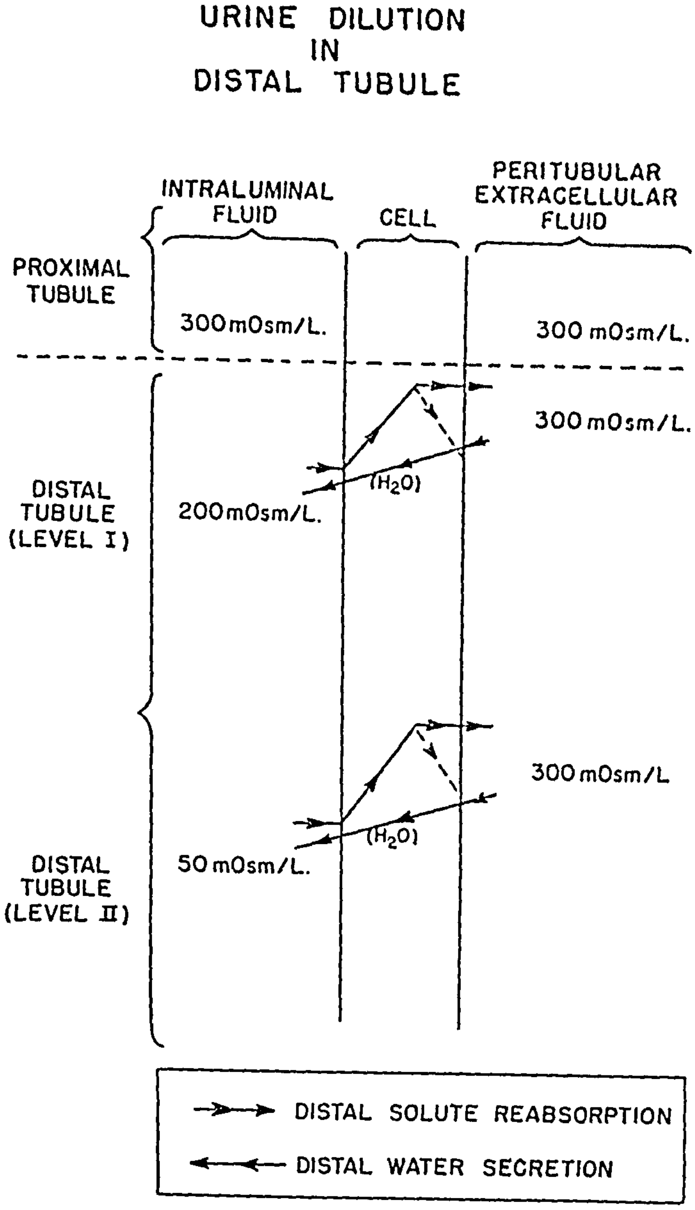

FIG. 3. SCHEMATIC COMPARISON OF SECRETORY AND REABSORPTIVE PROCESSES OF URINE DILUTION IN THE DISTAL TUBULE

It is assumed that the proximal and peri-tubular extracellular fluids are both isotonic with plasma, but hypertonic to the distal fluid. The assumed osmolarities are designated in the diagram. The tonicity of the final urine has been taken as ⅙ that of plasma. The lines pointing toward the left represent energy potentials for the process of secretion with the production of a fluid approaching water in composition. The lines pointing to the right represent energy potentials for an assumed process of distal solute reabsorption which requires a hypertonic reabsorbate. Two levels of the distal tubule, representing progressive stages of urine dilution, are portrayed. No definite value is assumed for the tonicity within the cell for the reabsorptive process. Alternative pathways from the cell to the extracellular fluid are drawn for the reabsorbate, solid lines representing a process of extrusion of a hypertonic fluid, and interrupted ones, a process of intracellular dilution.

level. Variations in the amount or in the mode of administration of water do not affect the pattern or extent of the diuresis, provided the dose is in excess of 1% of the body weight (15*b*). Even while water absorption in the intestine or injection by vein still continues (16*b*, 17), the typical decline of diuresis takes place. Throughout the entire course of water diuresis, the filtration rate remains essentially unchanged (18–20). Hormonal and nervous factors, *i.e.*, pituitrin, epinephrine, thyroxine, and painful or emotional stimuli, have profound effects on both the course and amount of the diuresis (21, 22).

This behavior resembles that of secretory glands. Their function is characterized by a specific pattern often rhythmical in nature (23, 24). The volume of secretion may vary independently of the blood flow (25). Nervous and hormonal factors profoundly affect their activity. Hypotonicity of secretions is well known, sweat and saliva being the outstanding examples (26).

Some of the thermodynamic implications of the production of hypotonic urine, according to the alternate assumptions of distal secretion of water or distal reabsorption of solutes, may be examined. Figure 3 is a schematic presentation of the assumed energy relations and fluid concentrations at two levels of the distal tubule. At any level in the distal tubule the concentration gradient between peri-tubular fluid and the secretion is 300 mOsm./L., while that between intra-luminal and peri-tubular fluids increases progressively. With respect to an assumed process of solute reabsorption, the less the volume of water reabsorbed, the more hypertonic must be the reabsorbate. No specific assumptions are available for the volume of fluid delivered to or reabsorbed from the distal tubule. For simplicity, a constant rate of solute reabsorption at all levels in the distal tubule has been assumed. Whatever volume of a hypertonic reabsorbate is postulated, its production will require more energy than that of a dilute secretion.[5]

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[5] The energy expenditure in the production of dilute urine by the distal tubule, assuming a single stage physical process, may be calculated according to the equation of von Rhorer (27).

$$1)\quad W = VRT\,(U \ln U/P + P - U)$$

The limitations of and justifications for such a method of calculation of osmotic work have been discussed in detail elsewhere (10).

The volume of water secreted or reabsorbate removed to

Inspection of the diagram reveals that the concentration gradient between lumen and reabsorbate at any level in the tubule is greater than that between peri-tubular fluid and a dilute secretion. Furthermore, the gradient increases progressively as the urine becomes more dilute. Whether the hypertonic reabsorbate *per se* is extruded into the peri-tubular space or whether it is diluted to isotonicity in the tubular cell, the process of distal solute reabsorption would seem uneconomical compared to that of distal secretion.

According to the postulate that there is a distal secretion, water is transported from an isotonic peri-tubular fluid to the tubular lumen. Such a process may be experimentally produced and is known as "negative anomalous osmosis." The factors responsible for negative osmosis depend chiefly on the characteristics of the membrane (28). A similar process of negative osmosis might result in movement of water from hypertonic distal to isotonic peri-tubular fluids, accounting for the concentration of urine in normal hydropenic subjects or in patients with diabetes insipidus when they receive pitressin. No fundamental change in membrane permeability would have to be assumed to account for either concentration or dilution of the urine by the kidney.

The large volume of glomerular filtrate renders difficult any attempt to establish the existence of a tubular secretory function. Perhaps the only acceptable direct evidence would consist of demonstration of a rate of urine flow in excess of that of filtration, under conditions of extreme reduction of filtration rate.

## SUMMARY

Osmotic diuresis was produced by mannitol loading in three patients with diabetes insipidus.

During hydropenia, a flow of copious and dilute urine persisted. After loading, osmolarity of the urine rose but remained below that of plasma while the urine flow increased more than two-fold. For a given solute load the rate of excretion of urine was two to three times that of normal subjects. The urine flow was independent of changes of filtration rate or blood flow. After administration of pitressin, the relation of urine flow and solute excretion became essentially normal.

The per cent composition of urinary solutes in patients with diabetes insipidus during mannitol loading was similar to that of normal subjects suggesting that the final solute load destined for excretion is assembled in a portion of the tubule proximal to the site of concentration or dilution of the urine.

The data on urinary flow and solute load indicate that there exists a basal, dilute urine flow in diabetes insipidus, and that the increased flow ob-

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dilute a given amount of isotonic proximal fluid may be calculated as follows:

It is easily seen that

$$2)\quad (O_p - O_u)n$$

is the number of solutes removed in the process of formation of dilute urine from an isotonic fluid, be it peri-tubular, extracellular or proximal tubular fluid, where $O_p$ = plasma osmolarity, $O_u$ urine osmolarity, and n the observed rate of urine flow. Then,

$$3)\quad \frac{(O_p - O_u)n}{O_p} = V_s,$$

the volume of a secretion of water added to a given amount of isotonic proximal fluid, and

$$4)\quad \frac{(O_p - O_u)n}{O_r} = V_r,$$

the volume of a reabsorbate of osmolarity $O_r$, removed from a given amount of isotonic proximal fluid.

It follows that,

$$5)\quad V_r = \left(\frac{O_p}{O_r}\right) V_s.$$

In the case of distal secretion, the concentration gradient is from peri-tubular to secretion fluid. Substituting in equation *1*), U is the concentration of the secretion, and P that of the peri-tubular fluid. In the case of solute reabsorption, the gradient is from distal fluid to reabsorbate, so that U would represent the assumed osmolarity of reabsorbate and P, that of the distal fluid. As a first approximation, the osmolarity of the secretion may be taken as zero. For the reabsorbate, a series of values in multiples of plasma osmolarity are assumed. The osmolarity of the peri-tubular fluid is taken to be 300, and the final urine, 60 mOsm./L. Substituting these values in equation *1*) gives a series of relative work units which permit a comparison of energy expenditure in terms of a given volume of isotonic fluid cleared of solutes as is shown in the following table:

TABLE III

*Energy expenditure for secretory and reabsorptive process in the distal tubule*

| Vol. of secretion | Osmol. peri-tub. fluid | Osmol. of secretion | Work units |
|---|---|---|---|
| *cc./min.*<br>$V_s$ | *m Osm./L.*<br>300 | *m Osm./L.*<br>0 | *W/RT*<br>300 $V_s$ |
| Vol. reabsorbate | Osmol. distal fluid | Osmol. reabsorbate | |
| $V_s/2$ | 60 | 600 | 420 $V_s$ |
| $V_s/3$ | 60 | 900 | 536 $V_s$ |
| $V_s/4$ | 60 | 1,200 | 615 $V_s$ |
| $V_s/10$ | 60 | 3,000 | 876 $V_s$ |

served during loading is due to the delivery of additional isotonic fluid from the proximal tubule.

It is postulated, on indirect evidence, that the distal tubule may be a special organ of water transport and that the production of hypotonic urine is due to water secretion, that of a hypertonic urine to water reabsorption, in the distal tubule.

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# FACTORS MAINTAINING CEREBRAL CIRCULATION DURING GRAVITATIONAL STRESS

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(Submitted for publication July 27, 1950; accepted, December 21, 1950)

## INTRODUCTION

The stresses developed during flight may make certain physiological mechanisms clear which would otherwise be difficult to demonstrate. This report is concerned with a condition induced by forces peculiar to high speed aircraft, but the results can be applied to the cerebral circulation during the more familiar stresses induced by gravity in the erect position. Amaurosis fugax, or aviator's blackout (1), occurs in normal men when they are exposed in the upright seated posture to centrifugal forces acting for more than five seconds in the direction head to buttocks. Commencing at 3 to 4 gravity units as a dimming of vision most noticeable in the peripheral field, at 4 to 5 gravity units it progresses to temporary blindness and finally, at slightly higher levels, unconsciousness occurs. Lambert (2) has shown that apparently identical changes in vision can be produced by air pressure on the eye balls. By sucking on the eyes with a special mask, he prevented blackout during high accelerations. His experiment confirmed the belief that the centrifugal forces induce blackout by causing a decrease in the arterial pressure at eye level. This pressure drop results from an increase in the effective weight of the blood which increases the effective length of the column above the heart. Since the pressure is proportional to the acceleration, at 5 gravity units the normal arterial pressure becomes inadequate, for it must then raise the blood the equivalent of five times the 30 cms. distance from the heart to the head. Lambert and Wood (3) have shown that the systolic pressure at eye level during exposures producing blackout is less than 20 mm. Hg, and they have observed that the pressure often falls temporarily to zero (4). Rossen, Kabat and Anderson (5) have shown that if the blood supply to the human brain is suddenly interrupted, consciousness is lost in six seconds. The circulation should, theoretically, be halted by an acceleration which reduces the mean arterial pressure at head level to near zero values. In spite of this, maintenance of consciousness has been reported during a blackout of three minutes duration on the human centrifuge (6). This continued alertness for periods of 30 seconds or more, in the face of a measured cerebral arterial pressure of 20 mm. Hg to 30 mm. Hg (7) is surprising. However, a survey of the clinical experiences with syncope also yielded extremely low values. Data from the observation of four different conditions were available (Table I), namely, clinical orthostatic hypotension (8–10); a phlebotomy followed by syncope (11); the use of a tilt table with amyl nitrite (12) and collapse following puncture of the brachial artery (13). The significant parameter in the assessment of blood flow is the mean pressure. Since this was not explicit in the clinical data, it was calculated from the systolic and diastolic pressures by adding ⅓ of the pulse pressure to the diastolic pressure. (Table I, Column M). To make these figures comparable with the data obtained on the centrifuge mean pressure at eye level was estimated by subtracting from it the hydrostatic pressure of the fluid column between the brain and the various measuring points (Table I, Column E). The recorded values for visual symptoms, ranged about 30 mm. Hg to 40 mm. Hg; those for syncope were approximately 20 mm. Hg.

These facts suggest that compensating factors are at work and that a study of cerebral circulatory competence during exposure to gravitational fields should be of interest. The nitrous oxide technique for the accurate measurement of cerebral blood flow in man requires a longer period than was available for applying the stress with the human centrifuge. However, since there is little storage capacity for oxygen in the small blood content of the brain tissue itself, changes of cerebral venous oxygen content will reflect circulatory changes occurring within relatively short time intervals. Kety and his associates (14) have pointed out that the reciprocal of the cerebral arterio-venous oxygen difference represents the ratio of cerebral blood flow to metabolic demand. Although it yields no information concerning either of these parameters separately, the cerebral A-V $O_2$ does constitute a measure of the sufficiency of the cerebral circulation. It was, therefore, decided to measure the cerebral venous and arterial oxygen saturation during acceleration.

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## METHODS

Arterial pressures at head level were determined by a strain gauge blood pressure transmitter (15) connected by rigid walled, incollapsible vinylite tubing to a Lindeman type cannula placed in the radial artery (16). The wrist was held at head level and the gauge was firmly attached to a cap held at eye level. Thus even if the subject slumped in his seat during the acceleration, there would be no movement between the gauge and the brain. There is only 5 mm. Hg difference between the mean pressures in the carotid and radial arteries of a supine man (17); therefore, it was felt that the pressure in the radial artery is a satisfactory measure of that in the cerebral vessels if due account is taken for hydrostatic effects. Since blood flow is a function of mean pressure, mean pressures were assessed. Planimetry of sample pulse curves confirms the accuracy of the calculations of mean pressure made by adding ⅓ of the pulse pressure to the diastolic pressure (18).

The internal jugular vein was punctured by a needle inserted just below the mastoid process, using a 19 gauge needle. The technique was that introduced by Myerson, Halloran, and Hirsch (19) and described by Kety (20) in his recent account of the quantitative determination of cerebral blood flow in man. It results in the placement of the tip of the needle in a region just below the jugular foramen where collapse of the vein is impossible due to its bony attachment. A 2 by 3 cms. brass plate was fastened to the neck by adhesive tape and an attached clamp tightened around the needle to prevent slip-

TABLE I

*Estimated mean cerebral blood pressure in mm. Hg during syncope*

| Author and Method | Cause | Case | Blood pressure (*mm. Hg*) | | | | Symptoms as described by authors |
|---|---|---|---|---|---|---|---|
| | | | S/D | M* | E* | ME* | |
| Ellis and Haynes (8), Riva Rocci | Orthostatic hypotension | 1 | 65/52 | 56 | 44 | 27 | Feeling of faintness to absolute syncope |
| | | 2 | 62/46 | 51 | 29 | | Blurring of vision; "all gone feeling" |
| | | 3 | 33/26 | 28 | 6 | | Buzzing; blurring of vision |
| | | 4 | 62/41 | 48 | 24 | | Dizziness |
| | | 5 | 75/56 | 62 | 40 | | Dizziness |
| | | 6a | 56/44 | 48 | 33 | | Marked dizziness |
| | | 6b | 42/30 | 34 | 16 | | Marked dizziness |
| Jeffers, Montgomery and Burton (9), Riva Rocci | Orthostatic hypotension | WL | 50/45 | 47 | 25 | 21 | Vertigo; fainting ++ |
| | | LM | 60/50 | 53 | 21 | | Vertigo; fainting +++ |
| | | TM | 42/38 | 39 | 17 | | Vertigo; fainting ++++ |
| Gregory (10), Riva Rocci | Orthostatic hypotension | 1a | 45/? | | | 23 | Fainting |
| | | 1b | 50/? | | | | Very dizzy |
| | | 1c | 55/40 | 45 | 23 | | Dizzy |
| Loman and colleagues (12), Tycos elastic manometer, carotid artery | Sudden tilting | | | 28 | 22 | 19 | Fainting |
| | Amyl nitrite | | | 22 | 16 | | Verge of syncope |
| McMicheal (11), Riva Rocci | Hemorrhage | | 58/? | 20 | <20? | | Syncope |
| Rushmer (13), Hamilton manometer, Riva Rocci | Neurogenic collapse | | 65/? | | <43 | | Dizziness |
| | | | 45/? | | <23 | | Unconsciousness |

* M Mean arterial pressure obtained by adding ⅓ of pulse pressure to the diastolic pressure.
E Mean arterial pressure at eye level calculated by allowing for the estimated hydrostatic pressure of the vertical column between gauge and eye.
ME Average pressure from E.

Table of data collected from various sources showing that the mean arterial pressure can fall below 30 mm. Hg before consciousness is seriously impaired, but that syncope develops at 20–25 mm. Hg.

ping during the centrifuge run. Samples could be taken rapidly during the run by a series of three-way stopcocks, to each of which was attached a syringe (20). The heparinized samples were chilled until submitted for analysis of oxygen content by the Van Slyke manometric method (21). The blood was drawn by an experimenter sitting beside the subject in the cab of the centrifuge. Runs were made at accelerations ranging from 1.0 to 4.5 g and the force was held constant for one to two minutes at each acceleration until the blood samples had been drawn. Three trained subjects were employed. Once the needles had been inserted they were at ease. Their normal resting blood pressures give objective confirmation of their relaxation. In two subjects, four series of experiments, involving prolonged acceleration, were made and the oxygen content of 13 samples of cerebral venous blood determined at the various accelerations. In three of these series the arterial blood pressure was determined at the same time. (The possibility of equipment failure with leakage of air into the jugular vein was not ignored. The dangers of a small aeroembolism are inappreciable and the observer in the cab watched the transparent tube leading to the needle. If air leakage developed the blood would disappear from the tube. This could be seen and the needle removed at once. In practice this emergency did not occur.)

Jugular pressures were recorded by a second strain gauge transmitter also held at eye level. Both systems were flushed with heparinized saline to prevent clotting. A rapid pressure rise following a Valsalva maneuver and the presence of pulsations, which persisted in spite of digital occlusion of the jugular vein during acceleration, assured the patency of the needle and its free communication with the intracranial sinuses.

In two subjects the oxygen saturation of the blood in the jugular vein was measured continuously by drawing it through a Kramer glass cuvette oximeter (22) by a mechanically actuated syringe (23). The oximeter was calibrated by passing blood of known saturation through it. Since all the samples were drawn at the same constant speed, the flow effect, characteristic of these instruments, could be disregarded.

Changes in venous oxygen saturation will only reflect the sufficiency of the cerebral blood flow provided the arterial saturation remains essentially undisturbed. Therefore, in control experiments the oximeter was connected to the arterial cannula. Arterial saturation was measured during typical centrifuge acceleration "runs" such as 4.5 g for 15 seconds. It fell from 98% to 93%. Since the onset of this change was delayed by more than 10 seconds, it occurred too late to affect venous oxygen saturation significantly during the period of the greatest depression of the saturation. This was during the first 10 seconds of the 15 second runs. As will be shown, the most striking result has been the maintenance of cerebral venous oxygen saturation in spite of the fall in arterial pressure induced by the gravitation. No correction has been made for a possible slight fall in arterial oxygen saturation during the higher, prolonged accelerations. However, the error resulting from this omission would only depress the apparent venous oxygen saturation and give an impression of failure of circulatory sufficiency.

During both arterial and venous oximetry, the blood accumulated in the mechanical syringe constituted an integrated sample giving the mean value of the saturation throughout the sampling period. There was a seven to eight second delay before the oximeter revealed changes in the oxygen content. This lag was due to the time taken for passage of the blood from the brain capillaries to the needle (three to four seconds) and thence to the cuvette (four seconds).

Light signals in the peripheral and central fields of vision were employed to assess the visual impairment during the runs. The responses were recorded photographically, together with the other variables under study.

## RESULTS

### 1. *Cerebral venous oxygen content in relation to mean arterial pressures*

In Figure 1 mean arterial blood pressure at head level is compared with mean venous oxygen saturation. The pressure fell in direct proportion to the acceleration, attaining 30 mm. Hg at approximately 4.0 g.[3]

The mean cerebral venous oxygen saturation stays materially unchanged in contrast with the mean arterial pressure. In fact, two of the three series actually show a slight rise in the 2 to 3 g range. Some fall occurs at higher accelerations but never to the critical values that would be expected in view of the fall in arterial pressure. Fast records of the behavior of the venous oxygen content during the acceleration show the same picture. Figure 2 is a typical example of three records taken of two of the subjects, showing changes in oxygen saturation in the jugular vein as recorded by direct

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[3] In extensive studies of arterial pressure at eye level during 15 second accelerations of abrupt onset, Lambert and Wood (3) have obtained similar results. The main difference is the acceleration at which they observed visual symptoms. They found that blackout normally occurs at 4 to 5 g and their data indicate that the lowest systolic pressure inducing a blackout during exposure was often zero, and generally below 20 mm. Hg. In the present study the mean arterial pressure, as measured throughout the run, was still approximately 30 mm. Hg at 4.5 g. Higher accelerations were needed to produce visual symptoms in these experiments because in order to give time for sampling, the centrifuge was accelerated gradually and the run lasted from one to two minutes. This technique elicits a full vasomotor response and consequently the arterial pressure was higher than in runs used by Wood and his associates (4).

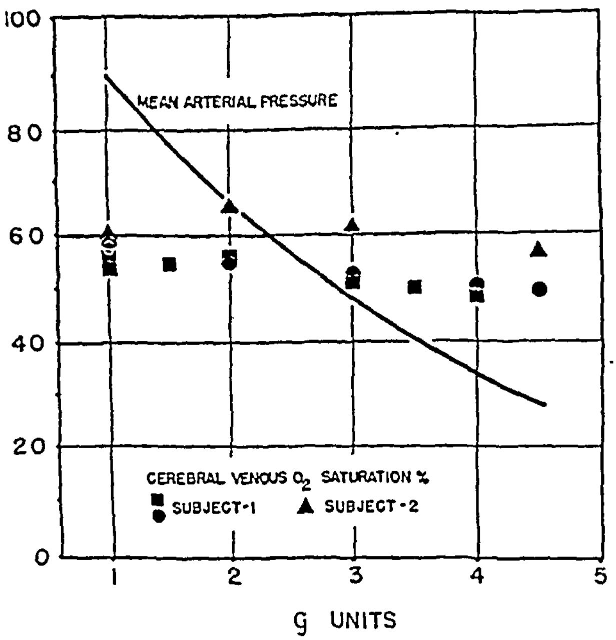

FIG. 1. MEAN ARTERIAL PRESSURE AT HEAD LEVEL PLOTTED AGAINST ACCELERATION

Ordinate: pressure in mm. Hg and venous oxygen saturation per cent. Abscissa; g units. Cerebral venous oxygen saturation remains essentially constant in spite of the fall in arterial pressure during the prolonged one to two minute accelerations.

oximetry. Events in the curve are delayed for seven to eight seconds by the time constants of the oximeter. After the initial increase from 50% to 55%, the saturation falls to 42% at the time when the arterial pressure is lowest. The vasopressor response is associated with a rise in venous saturation which follows the behavior of the blood pressure after the acceleration ends. However, these changes in oxygen saturation are small in comparison with the great fluctuations in arterial pressure at head level.

Since the arterio-venous oxygen difference is a measure of the sufficiency of the cerebral blood supply, some compensatory effect must be responsible for its remarkable constancy in face of the changes in arterial pressure. This effect could either be vasodilatation reducing cerebral vascular resistance or a fall in venous pressure increasing the force driving the blood through the capillary bed.

### 2. *Pressure Measurements in the Jugular Bulb*

It seemed possible that as a result of the acceleration, a negative pressure might develop in the intracranial veins which continuously sustained cerebral blood flow and, therefore, venous oxygen saturation, by partially compensating for the marked fall in cerebral arterial pressure. In addition to animal studies using the saggittal sinus as a pressure source (7), repeated measurements

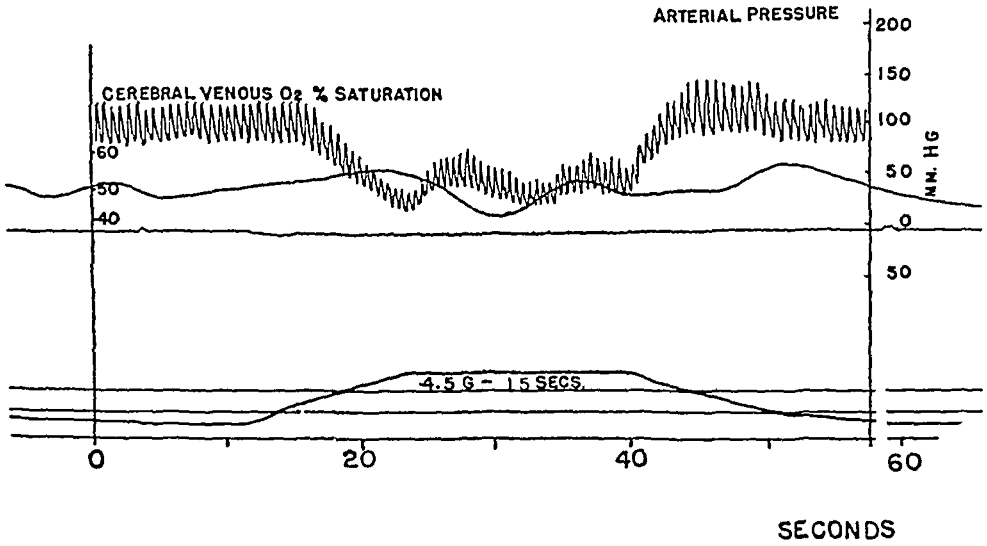

FIG. 2. ARTERIAL PRESSURE IN SUBJECT 3 AT HEAD LEVEL DURING A BRIEF 4.5 G ACCELERATION, LASTING 15 SECONDS

Abscissa; time in seconds. Ordinate; on left: cerebral venous oxygen saturation in volumes per cent; on the right: arterial pressure in mm. Hg. The constants of the oximeter cause a seven second delay in the response curve.

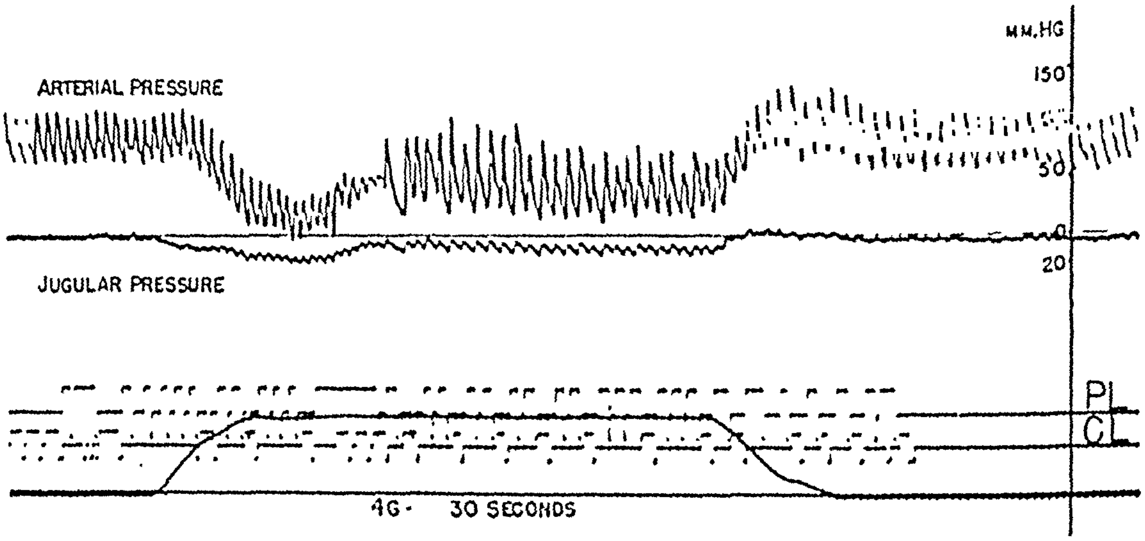

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FIG. 3. CEREBRAL ARTERIAL AND JUGULAR VENOUS PRESSURES DURING A 30 SECOND RUN AT 4.0 G
Peripheral lights (PL) and center lights (CL) responses respectively. Note the failure to respond to the peripheral lights for a few seconds after the deepest fall in arterial pressure.

were taken at the superior jugular bulb using three subjects submitted to both brief and prolonged accelerations. In each case the pressures remained subzero throughout the acceleration. Brief accelerations of abrupt onset gave the most marked pressure falls and records showing the highest and lowest values obtained with such runs are presented. In the third subject the results were intermediate but of an identical pattern. In one run the arterial and jugular pressures were recorded during a 30 second acceleration (Figure 3). Seven seconds after the initial fall in arterial pressure, the vasopressor response took effect and the arterial pressure recovered to some extent. The first and third interrupted lines below the venous pressure curve, respectively, register response to peripheral and central lights (24). This case lost his peripheral lights for 3.5 seconds at a mean arterial pressure

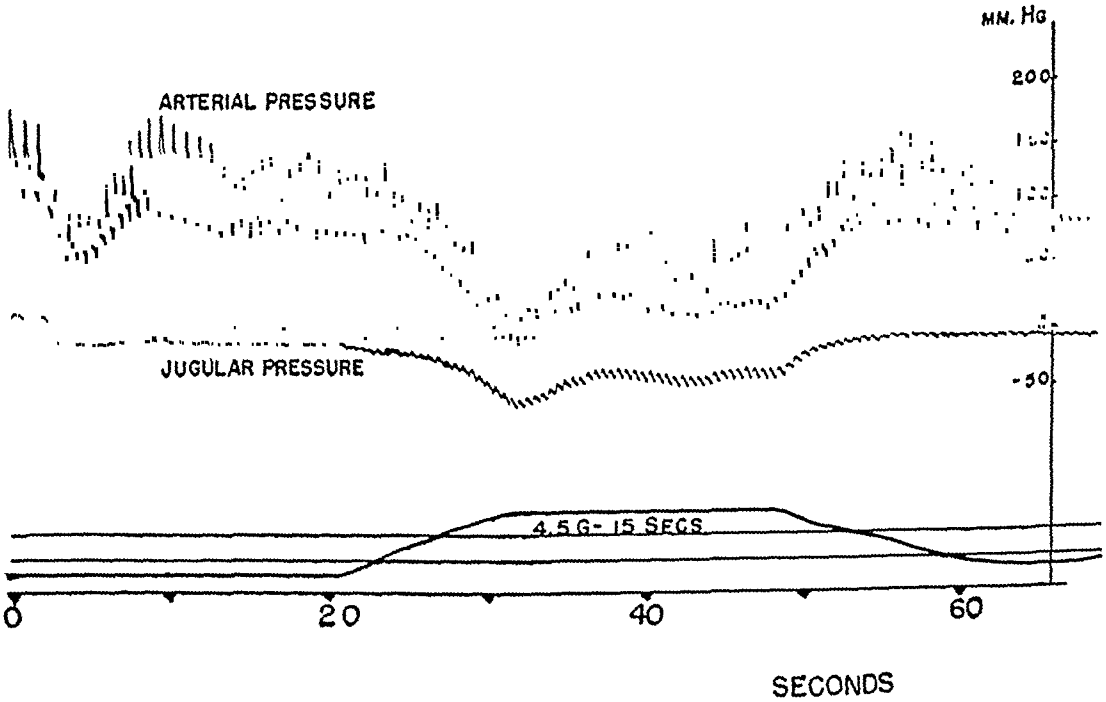

FIG. 4. CEREBRAL ARTERIAL AND JUGULAR VENOUS PRESSURES DURING A 4.5 G, 15 SECOND RUN, SHOWING THE CONTINUED RETENTION OF A HIGH ARTERIO-VENOUS PRESSURE DIFFERENTIAL DUE TO A MARKED FALL IN JUGULAR PRESSURE DURING THE ACCELERATION

of 10 to 15 mm. Hg, then during the vasopressor response, the mean arterial pressure rose to 50 mm. Hg and stabilized at 35 mm. Hg. The jugular pressure fell to 20 mm. Hg negative pressure at the time of the greatest fall in arterial pressure. Later, as the arterial pressure rose, the jugular pressure became less negative but still remained 8 mm. Hg less than ambient. At critical levels of cerebral arterial pressure, this addition could represent the difference between unconsciousness and adequate responses (see Table I).

In another case the jugular suction effect was much greater (Figure 4), and the fall was momentarily approximately 60 mm. Hg. Although the mean arterial pressure was less than 10 mm. Hg for three to four seconds, and less than 30 mm. Hg for more than six seconds, only mild visual dimming occurred. This may be because the venous pressure followed the arterial pressure changes and the cerebral arterio-venous pressure differential was never less than 50 mm. Hg. It was noted that whenever the onset of the acceleration was gradual, the magnitude of the jugular pressure fall was far less. A possible explanation for this will be advanced in the discussion.

## DISCUSSION

These experiments demonstrate that there is some mechanism which compensates for the fall in cerebral arterial pressure induced by gravitational stress. Scheinberg and Stead (18) have also observed that when tilting patients into the erect posture, the fall in arterial pressure induced by gravity stress was of greater extent than that of the cerebral blood flow. They too, point out that the apparent fall in cerebral vascular resistance could either be due to a mechanical pressure decrease on the venous side of the capillary bed, or to active cerebral vasodilation. An additional effect could be a passive dilation of the brain vessels brought about by a fall in venous pressure. It is possible that all of these mechanisms may be in operation at the same time.

When the stress is imposed rapidly, the ensuing subatmospheric pressures which have been demonstrated in the jugular bulb of men and the saggittal sinus of cats and dogs (25, 7) are produced almost instantaneously. Any active dilation mediated through the nervous system would require several seconds. Not only does the subatmospheric pressure effect develop rapidly, but also it is sufficiently intense to account for the continued blood flow and high venous oxygen saturation, in a large part. However, at no time is it large enough to completely balance the fall in arterial pressure. Even in the case shown in Figure 4, the arterio-venous pressure differential sinks approximately to one half of its normal value during the run. The fact that cerebral vascular sufficiency, as indicated by the cerebral arterio-venous oxygen difference, is hardly affected by such a marked fall in the driving pressure makes it probable that the flow is sustained by a decrease in cerebral vascular resistance which assists the direct pull of the venous pressure fall.

There is a purely mechanical means by which such a fall in resistance may be brought about by a pressure decrease on the venous side of the capillary bed. A drop in pressure in the venous plexus about the spinal cord during acceleration in the upright seated posture would lead to collapse of these veins by drainage into the lower abdominal system. This space left by them would be filled by cerebro-spinal fluid draining from the cranial cavity. Thus a passive distension of the cerebral vessels might occur to fill the gap left by the cerebro-spinal fluid. During the distension of the veins a small amount of blood will be aspirated from the arterial tree. It was this brief excess of blood flow that Ranke (26) and others (25) believed might play some part in preventing loss of consciousness during short-lasting accelerations. For it has long been known that venous and cerebro-spinal fluid pressure in the head becomes negative in man when he assumes the erect posture (27). Ranke (26), Jasper and Cypriani (28), and Rushmer, Beckman and Lee (25) also recognized that in animals these pressures become strongly subatmospheric during positive acceleration. It has not been generally realized that significant deep channels, which can remain patent in spite of subatmospheric pressures, are available for the return of blood from the brain. Nor, as a matter of fact, has the partition into the various available channels of the blood returning during acceleration yet been analyzed.

It is now appreciated that the internal and external vertebral venous plexes (29) are extensive and the internal system is protected from collapse during acceleration by the subatmospheric cerebro-spinal fluid pressure (25). Therefore, an uninterrupted flow can continue down these vessels. The fact that edema of the face has been reported following blocking of the superior vena cava suggests that in man the capacity of the subsidiary channels is limited and that extra pressure is needed to force the full cerebral blood flow through them (30). Thus any rise in arterial pressure, or active or passive vasodilatation tending to increase blood flow during acceleration would be partially offset by a rise in pressure in the jugular bulb. When the pressure in this region becomes atmospheric, then it may be assumed that the jugular veins become available and the resistance to flow would fall accordingly. Such a mechanism would explain the simultaneous fluctuations of arterial and venous pressures observed in these records. For, as arterial pressure fell and flow decreased, the pressure in the jugular bulb at the entrance to the cervical venous system would fall and the venous pull would increase correspondingly. It also explains why when the gravitational stress is imposed slowly and there is adequate time for a vasopressor response, the fall in jugular pressure is far less pronounced than during the first few seconds after an abrupt acceleration when the fall in cerebral arterial pressure is most marked.

The increased pulsation in the jugular tracing during the acceleration would suggest a vasodilatation (Figures 3 and 4). These waves do not originate from the auricle and are not transmitted up to the jugular vein, for they are not eliminated by manual occlusion of the vein during acceleration. They might be due to the direct transmission of the arterial pulse through passively dilated cerebral vessels. Alternatively, the venous pulsations may be due to direct transmission of volume changes from arteries to veins across the tissues of the brain. The pulsations would become more intense when the cerebral arterial pressure decreased during the acceleration because the arteries are not then so completely distended and would, therefore, fluctuate in volume more at any given pulse pressure.

There is some evidence of active cerebral vasodilatation during acceleration. Thus it was found that the cerebral venous oxygen saturation in anesthetized animals exposed to positive acceleration remained elevated for several minutes after the run in spite of normal arterial pressures (31). It is of interest in this connection that of the two 1 g controls in the series identified by the squares in Figure 1, the higher value of 57.2% was for blood taken immediately after a series of prolonged runs. However, data such as these can, at best, only be regarded as suggestive. Fortunately, Kety and his associates (14) have recently shown that when in hypertensives, the blood pressure is reduced by spinal sympathetic block, there is a partial relaxation of the cerebral vessels which tends to counteract the decrease in cerebral flow which would otherwise occur. This reflex relaxation does not compensate as fully for the fall in blood pressure as do the various mechanical and nervous factors at play during gravitational stress. This can be seen by comparing Figure 1 in their article with Figure 1 in the present article. A reduction of the arterial pressure to one half its normal value has less influence upon the venous oxygen saturation in the cases undergoing acceleration. When contrasted with the results of gravity induced cerebral hypotension, the data of Kety's group throw light upon the relative roles played by mechanical factors and by active cerebral vascular relaxation in the determination of cerebral blood flow, always provided that such relaxation also occurs in normal persons. Also the observation that relaxation of the cerebral vessels appeared to be independent of the degree of hypertension does suggest that a similar relaxation may occur in normotensive individuals suffering a sudden fall in blood pressure.

If the arterial pressure falls below the value necessary to force the blood to head level and hold open the carotid vessels and the cerebral vascular bed, then unconsciousness will occur no matter how perfect the compensation provided by suction and other factors. Animal experiments (31) have suggested that at high accelerations, when the pressure falls too low to fill the carotid arteries up to the level of the base of the brain, they collapse and flow through the carotid system ceases abruptly. Although in the dog the remaining flow through the pressure protected vertebral and spinal vessels may be adequate to maintain consciousness (5, 32), in man this is not so. Consequently, in contrast with the dog (31), in man consciousness is lost when arterial pressures at head level become subzero.

The fact that slightly higher blood pressures are required to maintain vision when protection against the effects of acceleration is afforded by pneumatic anti-G suits (33) may be due to the increase in central venous pressure and in cerebral-spinal fluid pressure produced by the inflation of such suits.

Ernstene and Blumgart (34) have suggested that one of the reasons for the orthopnoea of congestive heart failure might be an improvement in the cerebral circulation that resulted from the lowering of the cerebral venous pressure in the erect position. It is possible that some significance should be attached to this factor (35, 36). Certainly, the evidence just presented has shown that when a subject is placed in the erect position in a high gravitational field, the cerebral vascular sufficiency may remain undisturbed in spite of a great fall in arterial pressure. It is not yet clear whether this occurs solely as a direct result of the decrease in pressure on the venous side of the cerebral capillaries or whether passive vasodilatation also occurs due to an expansion of the cerebral vascular bed, but it is probable that whenever the acceleration persists for more than a few seconds, a combination of these effects develops, together with some active cerebral vasodilatation.

## SUMMARY

1. Consciousness is usually lost when the mean cerebral blood pressure falls to 25 mm. Hg, but during exposure to high accelerations such as those producing aviator's blackout, alertness may be retained in spite of such mean pressures.

2. Arterial and cerebral venous oxygen saturation and arterial pressure at head level and cerebral venous pressures were measured in three subjects during acceleration. The venous saturation remained almost unchanged in spite of great falls in cerebral arterial pressure. This suggested that some factor was maintaining cerebral blood flow.

3. Pressures ranging from 20 to 60 mm. Hg below ambient were found in the jugular bulb. This effect could maintain consciousness by sustaining the arterio-venous pressure differential, or by producing a combination of such an effect with passive cerebral vasodilatation.

4. There is also evidence that active cerebral vasodilatation may occur during prolonged exposure to gravitational stress in the erect posture.

## ACKNOWLEDGMENTS

The authors would like to express their appreciation to Mr. E. E. Martin for his participation in these experiments and to Dr. Earl Wood for his comments on the manuscript.

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# CORRELATION OF THE 24-HOUR RADIOIODINE UPTAKE OF THE HUMAN THYROID GLAND WITH THE SIX- AND EIGHT-HOUR UPTAKES AND THE "ACCUMULATION GRADIENT"

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(Submitted for publication November 2, 1950; accepted, December 21, 1950)

Techniques for the determination of thyroid function have become increasingly refined since the demonstration of the relation of basal oxygen consumption to thyroid activity 50 years ago. Newer methods include the determination of serum protein-bound iodine and various procedures using radioactive iodine. The latter include direct measurements of the accumulation in the thyroid gland of an administered dose of radioactive iodine (1), studies of urinary excretion (2), and observations of the rate of formation of serum protein-bound radioiodine (3). All observers agree that there is an elevated rate of accumulation of radioactivity in the thyroid gland in hyperthyroid, a depressed rate in myxedematous, and an intermediate rate in euthyroid subjects. This is true whether the uptake is measured directly over the gland or indirectly by excretion curves.

There is no standardized procedure at present among various institutions using radioactive iodine, but the general preference seems to be for techniques measuring the accumulation of radioiodine directly over the thyroid rather than by excretion curves. Probably the measurement of radioactivity in the thyroid gland 24 hours following the administration of $I^{131}$ (4) is most commonly used.

Astwood and Stanley (1) found that if the radioactivity in the thyroid is measured at frequent intervals following the administration of a tracer dose of $I^{131}$, the concentration in the thyroid is a linear function of the square root of time. The slope of the line expressing this linear relationship is constant for the first eight to 12 hours in euthyroid subjects and has been called the "accumulation gradient." By means of this technique it is possible to obtain rapidly an evaluation of thyroid function and it is quite valuable in determining the effect of various experimental procedures on the function of the thyroid gland. For example, if an antithyroid compound is administered following the initial determination of the gradient, the relative activity of the drug can be judged by the extent to which further accumulation of $I^{131}$ is inhibited (5). Conversely, the effectiveness of an injection of thyrotrophin can be judged by the increase which it produces in the gradient (6).

Two important objections have been raised to using the accumulation gradient as an index of thyroid function, however. In the first place, no physiologic significance has yet been discovered for the finding that the early course of radioiodine accumulation follows a parabolic curve. Secondly, Astwood and Stanley described their technique during the early development of accurate counting devices and were limited to a small, shielded, Geiger-Mueller counter which was placed in direct contact with the skin overlying the thyroid. Consequently absolute determinations of radioactivity in the thyroid could not be made and the values were recorded only as "counts per second per 100 microcuries of administered $I^{131}$." This made it impractical to utilize the observations of these investigators in other laboratories since the counting device would have to be duplicated exactly in order to obtain comparable data. Data expressing the radioactivity in the gland as a fraction of the administered dose can invalidate the second objection.

The present investigation was undertaken in an attempt to correlate the 24-hour uptake with other indices of thyroid function involving the use of $I^{131}$. All measurements were made with an adequately shielded Geiger-Mueller counter at a distance of 55 cm. from the neck. The absolute values obtained can thus be compared with those of other investigators. The accumulation gradient, 6-hour, 8-hour, and 24-hour uptakes were determined in the same individual and the other three functions then correlated separately with the 24-hour uptake.

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## MATERIALS AND METHODS

All subjects were healthy, young, clinically euthyroid volunteers, predominantly female technicians. Separate determinations of all four indices were made in 75 instances using 38 subjects. Twenty-eight subjects were employed more than once, none more than three times. Those studied more than once were given oral desiccated thyroid following the initial determination.

A shielded Sylvania gamma counter (GG-306) was so arranged that only the region directly surrounding the thyroid would be counted when the apparatus was in position. A Tracerlab Autoscaler was used to total the counts. A device was employed which aligned the aperture of the shielding over the center of the thyroid gland and which could also be arranged to support a flask containing $I^{131}$ at the same distance, when required. All counting was done at a distance of 55 cm. from the end of the Geiger-Mueller tube to the skin over the thyroid gland. Using the tube end-on the effective center of the counting volume is 6 cm. from the near end of the tube. The apparatus is to be described in detail in a future communication (7).

Approximately 50 microcuries of carrier-free $I^{131}$ were pipetted into either a small 50 cc. glass beaker or Erlenmeyer flask, diluted with 15–20 cc. of tap water, and the absolute radioactivity determined at a distance of 55 cm. from the gamma counter to the near surface of the vessel. The subject was thereupon given the solution to drink. This was followed by a rinsing of another 15–20 cc. of tap water to facilitate removal of the radioactivity from the flask, mouth, and pharynx. A single rinsing was sufficient to remove 99 per cent of the radioactivity from the flask.

The thyroid region of the subject was then counted at approximately hourly intervals during the day. Correction was made for background radiation and extrathyroidal $I^{131}$ by subtracting counts made over the lower thigh of the subject. The thigh count is somewhat less than the count contributed by the non-thyroidal tissues of the neck and this is therefore an undercorrection. The error thus introduced is not large, however, and is not significant except during the first hour or two after the tracer dose is given (8).

Since the counting was done at a distance of 61 cm. from the skin to the effective counting center of the Geiger-Mueller tube, small variations in the depth of the center of the thyroid gland from the skin would have little effect on the number of impulses reaching the tube. It seemed unnecessary, therefore, to make any correction for the estimated center of the thyroid gland since the radioactivity in the flask was also measured with its near surface 61 cm. from the effective counting center of the tube. The calculated radioactivity in the gland was increased somewhat by back scatter from the cervical region, but this would seem to be far less (9) than that described by Myant, Honour, and Pochin (10).

TABLE I

| x | Formula | S. D. | S. E. |
|---|---|---|---|
| Accumulation gradient | $y = 31.2x - 3.26x^2$ | ±0.141y | ±0.0164y |
| 6-hour uptake | $y = 1.67x$ | ±0.129y | ±0.0153y |
| 8-hour uptake | $y = 1.43x$ | ±0.134y | ±0.0160y |

The accumulation gradient was determined by plotting graphically the *per cent* of administered radioactivity present in the thyroid against the square root of the time in minutes following administration. The slope of this line was the numerical value of the accumulation gradient. The various gradients, 6-hour and 8-hour uptakes were then plotted, respectively, against the corresponding 24-hour uptakes.

## RESULTS

A close correlation was found to exist between the 24-hour uptake and all other functions. In the case of the 6-hour and 8-hour uptakes, simple straight line formulae were found to fit the data quite well. Since the spread was greater with the higher uptakes, the standard deviation and standard error were calculated as a fraction of the calculated quantity rather than as absolute values. A straight line could not be fitted to the data correlating the accumulation gradient and the 24-hour uptake, but a parabolic curve having the formula $y = 31.2x - 3.26x^2$ was found to fit quite pre-

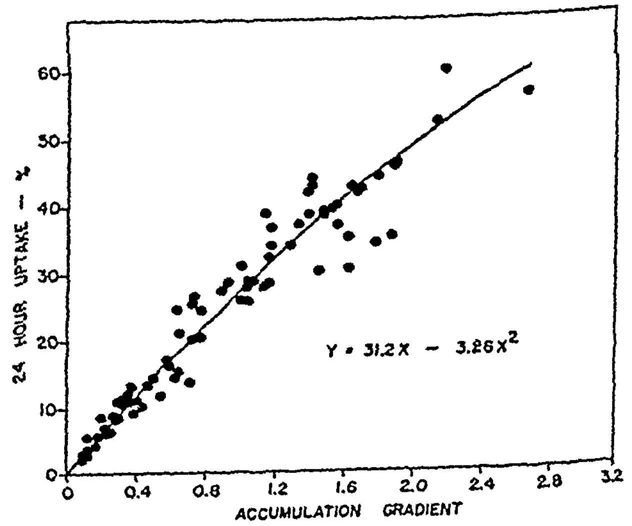

FIG. 1. RELATION OF ACCUMULATION GRADIENT TO 24-HOUR UPTAKE OF $I^{131}$ BY THE THYROID

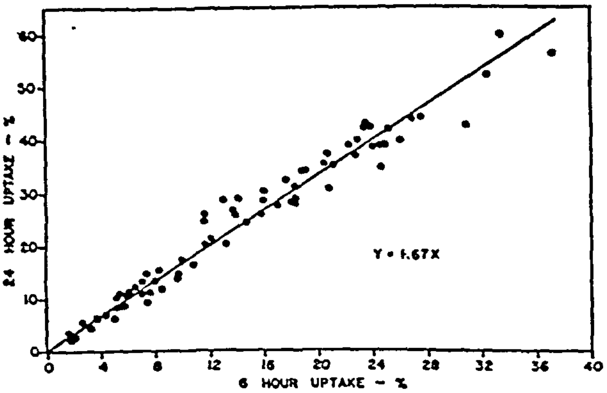

FIG. 2. RELATION OF 6-HOUR UPTAKE TO 24-HOUR UPTAKE OF $I^{131}$ BY THE THYROID

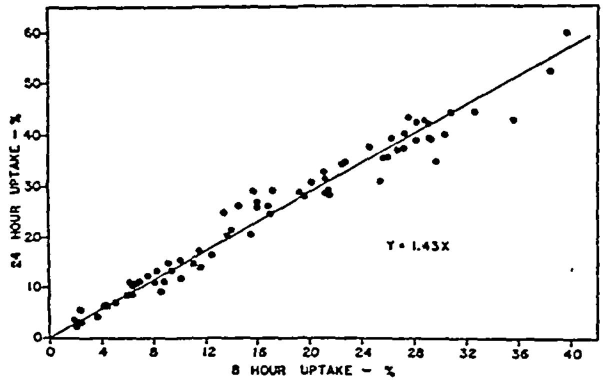

FIG. 3. RELATION OF 8-HOUR UPTAKE TO 24-HOUR UPTAKE OF $I^{131}$ BY THE THYROID

cisely. The formulae for the various correlations with the 24-hour uptake are listed in Table I. In each instance y represents the 24-hour uptake plotted on the ordinate and x represents the other functions, respectively, plotted on the abscissa. The formula for the accumulation gradient was calculated by the method of least squares. (Figures 1–3).

## DISCUSSION

The use of the data in this investigation makes possible the translation of the accumulation gradient, 6-hour, 8-hour, or 24-hour uptake in a euthyroid subject into comparable terms. Thus if a subject has an accumulation gradient of 1.6, using the formula $y = 31.2x - 3.26x^2$, the 24-hour uptake can be calculated to be 41.7 ± 6.9 per cent. The standard deviation of ± 6.9 is obtained by taking 14.1 per cent of y, in this instance 41.7 per cent.

The 24-hour uptake can thus be transformed into the 6-hour or 8-hour uptake by dividing by 1.67 or 1.43, respectively.

Such translations are chiefly of value in observations of normal or hypothyroid subjects. It is unlikely that the values obtained in thyrotoxic subjects would exhibit the same degree of correlation since the rate of uptake is often so rapid that the parabolic curve is followed for only a short time, making the gradient inaccurate. Also, some hyperthyroid patients exhibit a significant loss of activity from the gland following the first two to three hours after administration of $I^{131}$. This is presumably because a hyperactive gland contains only a small store of thyroxin which therefore is of unusually high specific activity. The higher rate of discharge in thyrotoxicosis serves further to accentuate this loss of radioactivity from the gland. In euthyroid subjects this does not occur since the organically bound $I^{131}$ serves to label a large "pool" of thyroxin and thyroglobulin in the colloid from which secretion takes place quite slowly. Consequently accurate predictions of the 24-hour counts from the accumulation gradients of thyrotoxic individuals are probably not possible.

The formula expressing the relation between the 24-hour uptake and the accumulation gradient is incorrect for the higher rates of accumulation seen in thyrotoxicosis. The parabola formed by this formula reaches a maximum at a gradient of 4.8. The corresponding 24-hour uptake is 75.8 per cent, obviously too low since many patients with severe hyperthyroidism have uptakes above 90 per cent.

The relatively large number of very low uptakes and gradients reported here for euthyroid subjects are due to the fact that these subjects had been given exogenous thyroid hormone as part of another study. It has been found that such therapy will produce a diminution in thyroid function, as measured by studies with $I^{131}$, to levels seen in myxedema (11). In every such instance, a previous uptake before thyroid medication had been instituted had shown a normally functioning gland. Although the activity of these thyroids was suppressed, no change was produced in the correlation of the various functions studied. It was of advantage to study subjects whose thyroid glands were suppressed to various degrees by exogenous hormone since in this manner many points could be calculated between very low and high uptakes, otherwise a difficult task.

## SUMMARY

The correlation of the accumulation gradient, 6-hour, and 8-hour uptake with the 24-hour uptake of radioactive iodine by the thyroid gland has been investigated in euthyroid human subjects. A close correlation has been found to exist between all four functions. If y is taken to represent the 24-hour uptake and x the various other functions, relatively precise formulae can be constructed by means of which the 24-hour uptake can be calculated from any of the other functions and vice versa. Thus, $y = 31.2x - 3.26x^2 \pm 0.141y$ is the formula for the accumulation gradient, $y = 1.67x \pm 0.129y$ is the formula for the 6-hour uptake, and $y = 1.43x \pm 0.134y$ is the formula for the 8-hour uptake. These correlations hold even when the thyroid gland is suppressed by exogenous thyroid hormone. The data presented here may be of value in future investigations of thyroid physiology in euthyroid subjects. It is suggested that far less correlation would be expected in thyrotoxic subjects.

## ACKNOWLEDGMENT

I am indebted to Dr. E. B. Astwood and Dr. J. F. Ross for helpful advice and suggestions in the preparation of this manuscript.

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# THE EFFECT OF THE UPRIGHT POSTURE UPON HEPATIC BLOOD FLOW IN NORMOTENSIVE AND HYPERTENSIVE SUBJECTS[1]

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(Submitted for publication, June 2, 1950; accepted, January 2, 1951)

## INTRODUCTION

The upright posture imposes a strain upon the cardiovascular system, chiefly as a result of its hydrostatic effects in the lower half of the body which, if unopposed, tend to produce hypotension in the upper half. These hydrostatic effects normally are counteracted by autonomic vasomotor adjustments, particularly in the splanchnic region. For example, the upright posture in man usually causes a reduction of renal blood flow (2). However, as yet no direct evidence has been reported concerning its effects upon the largest remaining portion of the splanchnic circulation, namely that draining through the liver. The present communication reports measurements of "hepatic blood flow" obtained with the bromsulfalein (BSP) extraction method (3) in normotensive and hypertensive subjects studied in the supine, in the upright, and again in the supine position.

## METHODS

The estimated hepatic blood flow (EHBF) was measured by the intravenous catheterization method of Bradley and associates (3). The continuous infusion of BSP was most easily maintained at a constant rate in both the horizontal and upright positions by suspending the infusion flask from a pole attached to the tilt table at such an angle that the flask remained at the same distance above the level of the subclavian vein when the table was horizontal or tilted up at 75°.

The subjects were hospital patients selected for study either because they had essentially normal cardiovascular systems or had arterial hypertension without evidence of cardiac, renal or hepatic insufficiency. All were studied in the basal state, at first supine, then tilted (passively) into the upright (75°) position for periods of 10 to 25 minutes (unless weakness and hypotension supervened), and finally, supine again. They were requested to remain as relaxed and motionless as possible throughout the test. Arterial pressure and pulse rate were measured either by the usual clinical methods or (in Cam, Aye and Dun) with a Hamilton manometer (4) at short intervals during each experiment.

Spot roentgenograms of the hepatic area were taken in each patient before, during, and after the upright tilt to demonstrate that the catheter remained properly placed in the same position near the center of the right lobe of the liver. To avoid possible errors due to mechanical and hydrostatic disturbances incident to changing the position of the subject, no result was computed on blood samples obtained within three minutes after a tilt, except for a few determinations made on blood samples that were taken immediately after a quick return to the horizontal position necessitated by faintness of the patient. As noted in Table I, such determinations have been related to the period just before rather than after this change of position because there appeared to be a time lag of one to two minutes before there was evidence of any acute change in hepatic blood flow as reflected in altered concentrations of BSP in the blood samples. After this time lag, however, considerable changes in serum BSP concentration could take place with great rapidity, especially when the subject had been tilted from the supine to the upright position. About four minutes after a tilt the trend of the BSP levels became more steady and the results after this time were accepted as more reliable measurements of an equilibrium state than those obtained earlier after a change in position. Furthermore, some subjects tolerated the upright position easily (top of Table IA and IB) and the results in them during upright tilting were regarded as more reliable than in the less stable subjects, some of whom fainted (bottom of Table IA and IB, and Figure 3).[4]

An effort was always made to draw the last pair of blood samples in each period as late as possible before a change in tilt table position. All estimates of rate of blood flow were calculated for the actual times of sampling and on the observed rather than the interpolated

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[1] Presented May 5, 1947 at the Thirty-Ninth Annual Meeting of the American Society for Clinical Investigation, Atlantic City, New Jersey (1).

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[4] Only those data obtained after four minutes in a given position, and without fainting, are included in the statistical analysis in Table II.

TABLE I

*Effects of the upright posture upon estimated hepatic blood flow (EHBF), mean (half systolic plus diastolic) arterial pressure, calculated hepatic-portal resistance (HPR) and pulse rate in normotensive (A) and hypertensive (B) subjects, arranged in order of their relative adaptability (freedom from symptoms and signs of poor postural response) to the upright posture*

| Patient Age Sex Surface area (*sq. m.*) | Horizontal position, Control period | | | | Upright (75°) position | | | | | Horizontal position, Recovery period | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | EHBF (*cc./min./ 1.73 sq. m.*) | Mean arterial pressure (*mm. Hg*) | HPR (*mm. Hg/ cc./sec./ 1.73 sq. m.*) | Pulse rate (*Beats/min.*) | Minutes after tilt-up | EHBF (*cc./min./ 1.73 sq. m.*) | Mean arterial pressure (*mm. Hg*) | HPR (*mm. Hg/ cc./sec./ 1.73 sq. m.*) | Pulse rate (*Beats/min.*) | Minutes after tilt-back | EHBF (*cc./min./ 1.73 sq. m.*) | Mean arterial pressure (*mm. Hg*) | HPR (*mm. Hg/ cc./sec./ 1.73 sq. m.*) | Pulse rate (*Beats/min.*) |
| | | | | | | | (A) | | | | | | | |
| Ema<br>39<br>M<br>1.70 | 1,924 | 98 | 3.1 | 68 | 4½ | 1,143 | 97 | 5.1 | 104 | 8½ | 1,858 | 100 | 3.2 | 72 |
| | 1,716 | 103 | 3.6 | 76 | 11 | 1,312 | 90 | 4.1 | 98 | 15½ | 1,713 | 103 | 3.6 | 74 |
| Ols<br>32<br>M<br>1.89 | 2,600 | 99 | 2.3 | 85 | 5 | 1,339 | 106 | 4.8 | 96 | 10 | 1,210 | 92 | 4.6 | 72 |
| | — | — | — | — | 13 | 1,375 | 102 | 4.5 | 90 | 20 | 1,155 | 91 | 4.7 | 84 |
| | — | — | — | — | 21 | 1,466 | 100 | 4.1 | 90 | — | — | — | — | — |
| Sil<br>25<br>M<br>1.98 | 1,762 | 101 | 3.4 | 100 | 4 | 1,319 | 99 | 4.5 | 120 | 5 | 1,381 | 95 | 4.1 | 102 |
| | 1,603 | 100 | 3.7 | 99 | 7 | 1,505 | 99 | 3.9 | 132 | 11½ | 1,900 | 95 | 3.0 | 96 |
| | — | — | — | — | 13 | 1,203 | 101 | 5.0 | 132 | 18 | 1,807 | 101 | 3.4 | 96 |
| | | | | | 18 | 1,381 | 104 | 4.5 | 138 | | | | | |
| Tef<br>29<br>M<br>2.08 | 1,500 | 106 | 4.2 | 80 | 5½ | 762 | 99 | 7.8 | 132 | 3 | 1,115 | 112 | 6.0 | 96 |
| | 1,411 | 105 | 4.5 | 80 | 13½ | 680 | 96 | 8.5 | 132 | 7 | 1,247 | 112 | 5.4 | 90 |
| | — | — | — | — | — | — | — | — | — | 14½ | 1,094 | 105 | 5.8 | 80 |
| Gio<br>24<br>M<br>1.78 | 1,107 | 98 | 5.3 | 86 | 6 | 50* | 78 | ? | 108 | 3½ | 944 | 97 | 6.2 | 92 |
| | 2,164 | 95 | 2.6 | 88 | 12½ | 796 | 91 | 6.9 | 108 | 19½ | 1,535 | 98 | 3.8 | 92 |
| | — | — | — | — | 21 | 978 | 89 | 5.5 | 104 | 31 | 1,034 | 99 | 5.8 | 84 |
| Car<br>24<br>M<br>1.76 | 1,101 | 103 | 5.6 | 88 | 5 | 818† | 88 | 6.5 | 114 | 3½ | 142* | 102 | ? | 102 |
| | 1,082 | 101 | 5.6 | 87 | — | — | — | — | — | 8½ | 874 | 98 | 6.7 | 82 |
| | — | — | — | — | — | — | — | — | — | 15½ | 912 | 100 | 6.6 | 84 |
| Fau<br>28<br>M<br>2.07 | 920 | 98 | 6.4 | 68 | 6½ | 703*‡ | 75 | ? | +? | 6½ | 962 | 97 | 6.1 | 58 |
| | 724 | 95 | 7.9 | 68 | — | — | — | — | — | — | — | — | — | — |
| McC<br>22<br>M<br>1.76 | 1,126 | 87 | 4.6 | 72 | 5½ | 1,174*‡ | 96 | 4.9 | 114 | 7½ | 644 | 89 | 8.3 | 63 |
| | 1,040 | 84 | 4.8 | 72 | 8 | 505 | ? | ? | ? | 13½ | 864 | 90 | 6.3 | 66 |

TABLE I—*Continued*

| Patient Age Sex Surface area (*sq. m.*) | Horizontal position, Control period | | | | Upright (75°) position | | | | | Horizontal position, Recovery period | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | EHBF (*cc./min./ 1.73 sq. m.*) | Mean arterial pressure (*mm. Hg*) | HPR (*mm. Hg/ cc./sec./ 1.73 sq. m.*) | Pulse rate (*Beats/min.*) | Minutes after tilt-up | EHBF (*cc./min./ 1.73 sq. m.*) | Mean arterial pressure (*mm. Hg*) | HPR (*mm. Hg/ cc./sec./ 1.73 sq. m.*) | Pulse rate (*Beats/min.*) | Minutes after tilt-back | EHBF (*cc./min./ 1.73 sq. m.*) | Mean arterial pressure (*mm. Hg*) | HPR (*mm. Hg/ cc./sec./ 1.73 sq. m.*) | Pulse rate (*Beats/min.*) |
| | | | | | | | (B) | | | | | | | |
| McC<br>43<br>M<br>1.75 | 1,624<br>1,503<br>— | 183<br>180<br>— | 6.8<br>7.2<br>— | 68<br>62<br>— | 3<br>8<br>18½ | 1,450<br>1,211<br>1,461 | 179<br>180<br>185 | 7.4<br>8.9<br>7.6 | 80<br>84<br>86 | 3½<br>13½<br>23½ | 1,380<br>1,591<br>1,844 | 193<br>188<br>190 | 8.4<br>7.1<br>6.2 | 68<br>64<br>65 |
| She<br>51<br>M<br>2.02 | 961<br>1,298<br>— | 131<br>142<br>— | 8.2<br>6.6<br>— | 64<br>67<br>— | 6<br>16<br>24½ | 702<br>979<br>760 | 141<br>137<br>141 | 12.0<br>8.4<br>11.0 | 84<br>87<br>88 | 12<br>20<br>— | 949<br>892<br>— | 134<br>130<br>— | 8.5<br>8.7<br>— | 64<br>64<br>— |
| Cam<br>51<br>M<br>1.71 | 1,118<br>—<br>— | 167<br>—<br>— | 9.0<br>—<br>— | 70<br>—<br>— | 6<br>11<br>16 | 1,072<br>1,059<br>998 | 175<br>171<br>170 | 9.8<br>9.7<br>10.0 | 84<br>88<br>83 | 5<br>10½<br>— | 1,036<br>900<br>— | 156<br>—<br>— | 9.0<br>—<br>— | 60<br>—<br>— |
| Val<br>37<br>M<br>2.05 | 1,217<br>1,440<br>— | 162<br>152<br>— | 8.0<br>6.3<br>— | 76<br>70<br>— | 3<br>7½<br>14½ | 566<br>825<br>969 | 174<br>165<br>163 | 18.0<br>12.0<br>10.0 | 90<br>112<br>108 | 3<br>10½<br>17 | 768<br>1,224<br>1,141 | 152<br>170<br>160 | 12.0<br>8.3<br>8.4 | 72<br>84<br>75 |
| Max<br>43<br>M<br>2.21 | 1,466<br>1,590<br>— | 133<br>130<br>— | 5.4<br>4.9<br>— | 56<br>60<br>— | 7<br>12<br>20½ | 876<br>713<br>694 | 127<br>128<br>131 | 8.7<br>11.0<br>11.0 | 72<br>84<br>? | 4½<br>14<br>24 | 2,650§<br>3,164§<br>2,050 | 136<br>143<br>133 | 3.1<br>2.7<br>3.9 | 66<br>60<br>60 |
| Ric<br>33<br>M<br>1.91 | 1,077<br>1,312 | 174<br>176 | 9.7<br>8.0 | 68<br>68 | 6<br>8 | 859<br>586 | 184<br>161 | 13.0<br>16.0 | 115<br>120 | 7<br>18 | 1,575<br>1,347 | —<br>— | —<br>— | —<br>— |
| Dea<br>54<br>M<br>1.82 | 1,462<br>1,227 | 159<br>177 | 6.5<br>8.6 | 58<br>64 | 6<br>10 | 1,154<br>1,041 | 164<br>179 | 8.5<br>10.0 | 66<br>69 | 10½<br>22½ | 1,363<br>1,444 | 168<br>168 | 7.4<br>7.0 | 60<br>56 |

TABLE I—*Continued*

| Patient<br>Age<br>Sex<br>Surface area<br>(*sq. m.*) | Horizontal position, Control period | | | | Upright (75°) position | | | | | Horizontal position, Recovery period | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | EHBF§ (*cc./min./ 1.73 sq. m.*) | Mean arterial pressure (*mm. Hg*) | HPR (*mm. Hg/ cc./sec./ 1.73 sq. m.*) | Pulse rate (*Beats/min.*) | Minutes after tilt-up | EHBF (*cc./min./ 1.73 sq. m.*) | Mean arterial pressure (*mm. Hg*) | HPR (*mm. Hg/ cc./sec./ 1.73 sq. m.*) | Pulse rate (*Beats/min.*) | Minutes after tilt-back | EHBF (*cc./min./ 1.73 sq. m.*) | Mean arterial pressure (*mm. Hg*) | HPR (*mm. Hg/ cc./sec./ 1.73 sq. m.*) | Pulse rate (*Beats/min.*) |
| (B)—*Continued* | | | | | | | | | | | | | | |
| Gol<br>31<br>M<br>1.81 | 1,095<br>980 | 179<br>177 | 9.8<br>11.0 | 84<br>78 | 6<br>— | 840<br>— | 158<br>— | 11.0<br>— | 90<br>— | 4½<br>9<br>14½<br>19½ | 802<br>1,123<br>1,215<br>1,168 | 174<br>175<br>176<br>176 | 13.0<br>9.4<br>8.7<br>9.0 | 72<br>72<br>75<br>75 |
| Pau<br>36<br>M<br>1.84 | 1,964<br>—<br>— | 124<br>—<br>— | 3.8<br>—<br>— | 66<br>—<br>— | 5½<br>—<br>— | 1,125<br>—<br>— | 123<br>—<br>— | 6.6<br>—<br>— | 90<br>—<br>— | 8<br>13<br>23 | 1,895<br>1,505<br>1,750 | 119<br>114<br>126 | 3.8<br>4.5<br>4.3 | 60<br>64<br>75 |
| Aye<br>51<br>M<br>1.86 | 1,409<br>1,408 | —<br>164 | —<br>7.0 | —<br>72 | 5½<br>— | 863†<br>— | 100<br>— | 7.0<br>— | 62<br>— | 6<br>11½<br>21½ | 772<br>928<br>916 | 120<br>125<br>131 | 9.3<br>8.1<br>8.6 | 58<br>56<br>53 |
| Dun<br>30<br>M<br>1.82 | 1,067 | 115 | 6.5 | 84 | 3 | 658† | ? | ? | ? | 4<br>12½ | 750<br>953 | 124<br>104 | 9.9<br>6.5 | 81<br>90 |
| Col<br>28<br>M<br>1.72 | 1,039<br>864 | 174<br>173 | 10.0<br>12.0 | 90<br>90 | 4½<br>— | 740‡<br>— | 102<br>— | 8.3<br>— | 61<br>— | 3<br>7<br>14½<br>24<br>37 | 752<br>808<br>1,478<br>894<br>1,164 | 139<br>170<br>172<br>170<br>169 | 11.0<br>13.0<br>7.0<br>11.0<br>8.7 | 72<br>82<br>84<br>90<br>96 |

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* This value was obtained during a rapid rise (more than 0.0006 mg./cc./min.) in the peripheral venous serum concentration of BSP. It is questionable, and is deleted from the statistical analysis (Table II).

† Patient faint, samples for EHBF taken, and immediately tilted back horizontal.

‡ Patient faint, tilted back horizontal, and samples for EHBF taken immediately.

§ Patient had severe headache and excitement.

values for serum concentration of BSP. Rate of change ($\Delta P$) in the peripheral venous or (in Cam, Aye and Dun) arterial concentration of BSP was taken as the calculated average for the preceding period in each instance. This value never exceeded 0.0006 mg./cc./min., except in four instances when the subject had been hypotensive[5] (Table I). Blood volumes were calculated by the method of Gibson and Evans (5). All estimates of hepatic blood flow were corrected for standard surface area (1.73 sq.m.). Hepatic portal resistance (HPR) was calculated simply by dividing the "mean" (one-half systolic plus diastolic) arterial pressure by the EHBF per second.

A cold pressor test (6)—immersion of one hand in ice water for one minute—was applied to six (four normotensive and two hypertensive) subjects in the horizontal position after a period of recovery from the effects of the upright position. This was abandoned later because it was found to produce only equivocal effects upon EHBF.

## RESULTS

A detailed presentation of data is given in Table I and a statistical summary in Table II. In both normotensive subjects (Table IA, Figure 1) and hypertensive patients (Table IB, Figure 2) the EHBF decreased following the tilt from the horizontal to the upright position. The decreases in EHBF were usually not associated with proportionate changes in mean arterial pressure, so that calculated hepatic-portal resistance (HPR) increased. Comparing only equilibrium values (Table II) the EHBF (cc./min.) decreased on the average from 1,713 to 1,070 in the normotensive group, from 1,357 to 960 in the hypertensive group, and from 1,499 to 1,004 in the combined groups. During the upright period, the average arterial pressure did not change, so that the HPR increased from 3.8 to 5.5 in the normotensive group, from 7.3 to 10.1 in the hypertensive group, and from 6.0 to 8.3 in the combined groups. These changes were significant statistically.

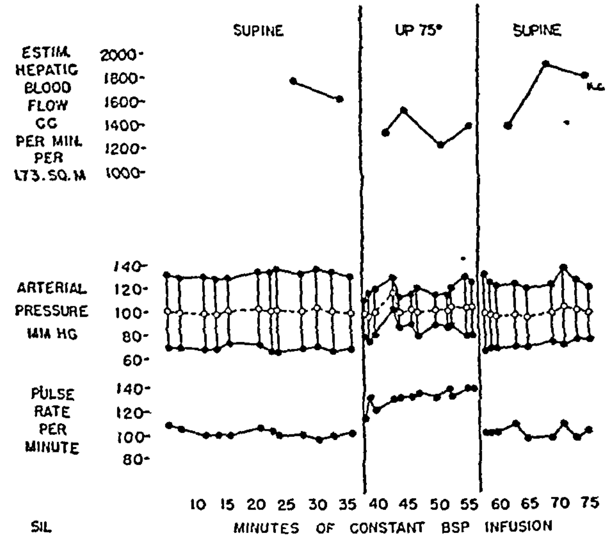

FIG. 1. CHART OF EHBF, ARTERIAL PRESSURE AND PULSE RATE IN A NORMOTENSIVE SUBJECT (SIL), IN THE HORIZONTAL, UPRIGHT, AND AGAIN IN THE HORIZONTAL POSITION

"Mean" arterial pressure (half systolic plus diastolic) is indicated by the open circles and interrupted line. At the first vertical line, the subject was tilted upright (75°) and at the second, horizontal again.

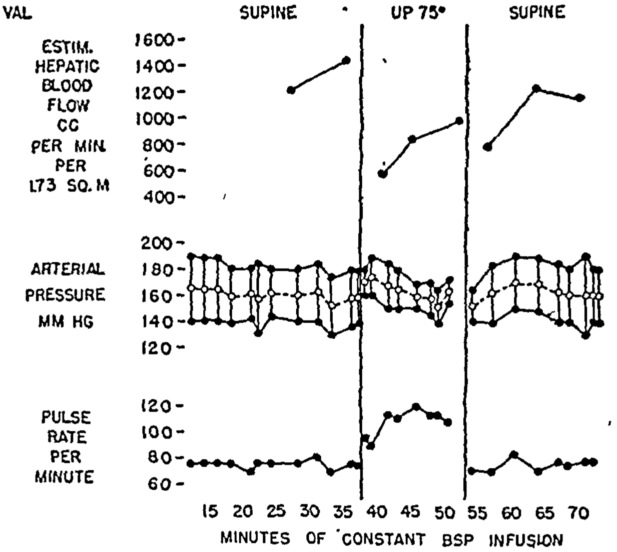

FIG. 2. CHART OF EHBF, ARTERIAL PRESSURE AND PULSE RATE IN A HYPERTENSIVE PATIENT (VAL)

Other notations as in Fig. 1.

The changes in EHBF and HPR were not directly related to increases in pulse rate or to other signs and symptoms of poor circulatory adaptation to the upright posture (Table I). However, there was in general a correlation between the tendency of a subject to collapse and to have a low EHBF in the upright position. Thus, of the six subjects who fainted, four had an EHBF below 750 cc./min. during the upright period and another had it just after being tilted back horizontal. Although six of the ten subjects who had an upright EHBF below 750 cc./min. did not faint, three (Gio, She, Val) of them had the low flow only during the first six minutes after being tilted up, and on further standing had an EHBF above 750 cc./min.

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[5] These data were omitted from the statistical analysis in Table II.

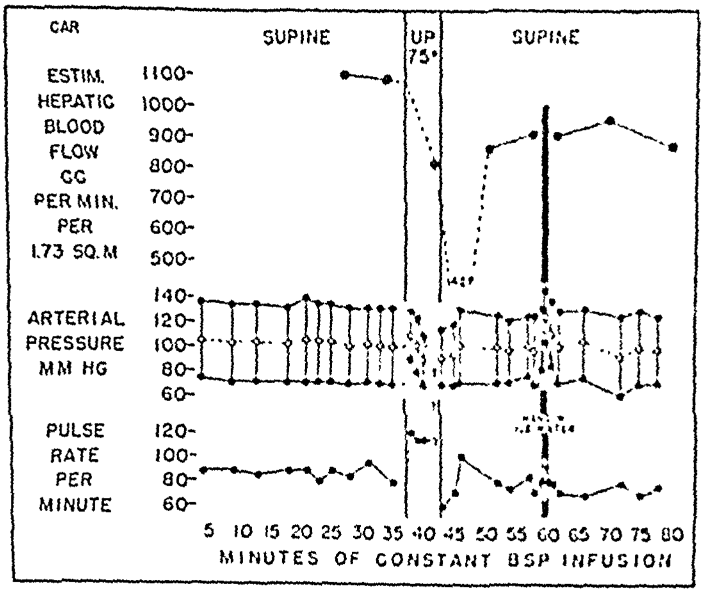

FIG. 3. CHART OF EHBF, ARTERIAL PRESSURE AND PULSE RATE IN A NORMOTENSIVE PATIENT (CAR)

At the heavy vertical line the left hand was immersed in ice water for one minute. Other notations as in Fig. 1.

Upon returning the subjects from the upright to the horizontal position the EHBF and HPR usually returned toward, to, or even beyond the control horizontal levels after varying periods of time. In some subjects the returns were prompt, in others they were delayed or incomplete, or occurred only after an initial overshoot beyond the previous horizontal values. In one hypertensive individual (Max) the post-tilt increases of EHBF and decreases of HPR were extreme and associated with a marked anxiety reaction that began with a severe headache.

Upon tilting the subjects back to the horizontal position the average values (Table II) changed as follows: EHBF rose from 1,070 to 1,312 in the normotensive group, from 960 to 1,417 in the hypertensive group, and from 1,004 to 1,372 in the combined groups, while the HPR decreased from 5.5 to 4.9, from 10.1 to 7.2, and from 8.3 to 6.2, respectively. Since the measurements during recovery showed greater variability than before tilting, the changes during this period, while significant for the combined and hypertensive groups, were not for the normotensive group considered separately.

Except in one patient (Sil), immersion of a

TABLE II

*Mean values* of estimated hepatic blood flow (EHBF), arterial pressure and hepatic portal resistance (HPR) in relation to posture, with a statistical analysis of the significance of the differences†*

| | EHBF (*cc./min./1.73 sq. m.*) | | | Mean§ arterial pressure (*mm. Hg*) | | | HPR (*mm. Hg/cc./sec./1.73 sq. m.*) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Horizontal (Control) | Upright | Horizontal (Recovery) | Horizontal (Control) | Upright | Horizontal (Recovery) | Horizontal (Control) | Upright | Horizontal (Recovery) |
| *Normotensives* (n = 6) | | | | | | | | | |
| Mean | 1,713 | 1,070 | 1,312 | 98 | 97 | 98 | 3.8 | 5.5 | 4.9 |
| Standard Error of Mean | 65 | 37 | 155 | 3 | 2 | 3 | 0.1 | 0.6 | 0.6 |
| Mean Difference‡ | — | −642 | +242 | — | −1 | +1 | — | +1.7 | −0.6 |
| Significance of Difference (P) | — | <0.01 | 0.12 | — | 0.75 | 0.81 | — | <0.03 | 0.42 |
| *Hypertensives* (n = 9) | | | | | | | | | |
| Mean | 1,357 | 960 | 1,417 | 158 | 157 | 155 | 7.3 | 10.1 | 7.2 |
| Standard Error of Mean | 97 | 68 | 172 | 7 | 7 | 8 | 0.6 | 0.7 | 0.7 |
| Mean Difference | — | −397 | +428 | — | −1 | 0 | — | +2.8 | −2.4 |
| Significance of Difference (P) | — | <0.01 | <0.05 | — | 0.78 | 0.97 | — | <0.01 | <0.01 |
| *Combined Groups* (n = 15) | | | | | | | | | |
| Mean | 1,499 | 1,004 | 1,372 | 134 | 133 | 131 | 6.0 | 8.3 | 6.2 |
| Standard Error of Mean | 107 | 61 | 116 | 9 | 9 | 9 | 0.6 | 0.8 | 0.6 |
| Mean Difference | — | −495 | +348 | — | −1 | 0 | — | +2.3 | −1.6 |
| Significance of Difference (P) | — | <0.01 | <0.01 | — | 0.77 | 0.87 | — | <0.01 | <0.01 |

* These data represent the group means calculated from the average values of EHBF, arterial pressure and HPR for each patient, shown in detail in Table I. Non-equilibrium observations (*i. e.*, those prior to four minutes after a change in posture, as well as those associated with a faint) were deleted from the calculations.

† The significance of the differences was calculated by Fisher's method for the significance of unique samples (12). P represents the probability that a difference may be due to chance. Values of 0.05 or less (in bold type) denote a "significant" difference, and 0.01 or less a "highly significant" one.

‡ The differences refer to the change from the immediately preceding posture. Slight discrepancies between the mean changes from upright back to horizontal as compared to the mean values in these positions are due to the fact that observations were made on one hypertensive patient (Ric) during the control and upright periods, but not during recovery.

§ Average of the systolic and diastolic pressures.

hand in ice water for one minute caused slight or negligible effects upon EHBF, although it usually caused a temporary elevation of arterial pressure (Figure 3) and HPR. It was felt that this stimulus, despite its severity, was too brief in duration to produce regular effects upon EHBF; consequently, it was abandoned.

## DISCUSSION

The results reported above confirm the impression gained from indirect evidence that active vasoconstriction occurs in splanchnic organs in addition to the kidneys when human subjects are tilted into the upright position. The decreases in EHBF found in the upright position can not be explained by changes in arteriovenous pressure gradient because the arterial pressure did not decrease proportionately and the atrial venous pressure is known not to increase in this position (7). Neither can the decreases in EHBF be attributed to other passive hydrostatic effects such as changes in blood volume (8) or intra-abdominal pressure (9). That the decreases in EHBF in the upright position are probably due to active vasoconstriction of sympathetic nervous origin has been indicated by repeating the studies of EHBF in some of the same subjects after splanchnicectomy (10).

No consistent differences in EHBF were found between the small group of hypertensive patients here reported and a larger group of normotensive subjects (11). The data indicate that the splanchnic circulation is not increased in hypertensive patients, but that calculated HPR is increased, reflecting the generalized vascular hypertonus usually regarded as characteristic of the disease. More evidence is being accumulated concerning these points by comparing the EHBF and HPR in the resting horizontal position in larger groups of hypertensive and normotensive subjects and will form the subject of a later report (11).

## SUMMARY

Hepatic blood flow, as estimated by the bromsulfalein extraction method, decreased in eight normotensive and 12 hypertensive subjects after passive tilting into the upright (75°) position. It failed to change regularly in six subjects after immersion of a hand in ice water for one minute.

## ACKNOWLEDGMENTS

The authors gratefully acknowledge the statistical analysis by Dr. Meyer H. Halperin, the technical assistance of Miss Adele Rymut and Miss Doris Bullard, and the photographic work of Miss Jane Holbrook.

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# THE EFFECT OF SPLANCHNIC SYMPATHECTOMY IN HYPERTENSIVE PATIENTS UPON ESTIMATED HEPATIC BLOOD FLOW IN THE UPRIGHT AS CONTRASTED WITH THE HORIZONTAL POSITION[1]

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(Submitted for publication June 2, 1950; accepted, January 2, 1951)

## INTRODUCTION

It has been found that when normotensive or hypertensive subjects are tilted passively into an upright position estimated hepatic blood flow (EHBF) (2) decreases and calculated hepatic portal resistance (HPR) increases (3). These changes were assumed to be due to active splanchnic vasoconstriction, probably mediated over the splanchnic sympathetic nervous system. Direct evidence bearing on this assumption became available in some of the hypertensive patients undergoing splanchnic sympathectomy and subsequently studied by the same methods as before operation. Such studies are the basis of the present report.

## METHOD

The method was identical with that used in the preoperative studies already reported (3). The first postoperative study was usually done within two weeks after the completion of the second stage of the splanchnicectomy, and, if possible, a second within 12 months. In addition, a few patients were studied one to nine years after (but not before) operation. The type of sympathectomy, unless otherwise noted in Table I, was the lumbodorsal (thoracolumbar) splanchnicectomy of Smithwick (4). After operation some patients[3] were unable to stand for any considerable length of time at a 75° tilt without marked postural hypotension and symptoms of faintness. Therefore, they were tilted back either to less marked angles (as noted in Table I) that they could tolerate long enough for reliable observations to be made, or to the horizontal position where paired samples of blood were drawn immediately for measurement of EHBF which, because of the time lag previously found to occur in this situation (3), was related to the upright period just before the tilt-back.

## RESULTS

After lumbodorsal splanchnicectomy postural hypotension occurred to some extent in almost all the patients, particularly early after the operation. If it appeared suddenly it resulted not only in less accurate measurements of arterial pressure (which varied from moment to moment as the patient sighed or moved), but also in other technical difficulties, since it made it necessary hurriedly to terminate the period of upright posture, rather than leisurely to proceed until a more steady state of EHBF had been achieved. For these reasons the results in these instances were considered less reliable as equilibrium values than those observed when the patient was able to stand without great difficulty for a considerable period of time.[4]

In all cases after splanchnicectomy there were more nearly proportional decreases of EHBF and mean (one-half systolic plus diastolic) arterial pressure in the upright position (Table I) than before operation when relatively large reductions of hepatic blood flow and small changes of arterial pressure were usually found (Table II). Therefore, after operation calculated hepatic portal resistance (HPR) was often unchanged in the upright position whereas before operation it was usually increased. Thus, comparing average equilibrium figures, HPR in eight splanchnicectomized hypertensive patients increased on tilting from 6.8 to only 7.2, or 6% (which is not significant statistically), whereas in nine unoperated hy-

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[1] Presented in part May 5, 1947 at the Thirty-Ninth Annual Meeting of the American Society for Clinical Investigation, Atlantic City, New Jersey (1).

[2] Public Health Service Special Research Fellow of the National Heart Institute. Present address—University Hospitals, Iowa City, Iowa.

[3] No patient was allowed to wear his abdominal pressure girdle or elastic stockings during the test.

[4] Only those data obtained after four minutes in a given position and without fainting are included in the statistical analysis in Table II.

TABLE I

*Effects of the upright posture upon estimated hepatic blood flow (EHBF), mean (half systolic plus diastolic) arterial pressure, calculated hepatic portal resistance (HPR) and pulse rate in splanchnicectomized hypertensive patients, listed in order of the relative completeness of the sympathectomy, as judged by anatomic and physiologic criteria* (4–6)

| Patient Age Sex Surface area (*sq. m.*) | Time after operation | Horizontal position, Control period | | | | Upright position | | | | | | Horizontal position, Recovery period | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | EHBF (*cc./min./1.73 sq. m.*) | Mean arterial pressure (*mm. Hg*) | HPR (*mm. Hg/cc./sec./1.73 sq. m.*) | Pulse rate (*Beats/min.*) | Minutes after tilt-up | Degree of tilt | EHBF (*cc./min./1.73 sq. m.*) | Mean arterial pressure (*mm. Hg*) | HPR (*mm. Hg/cc./sec./1.73 sq. m.*) | Pulse rate (*Beats/min.*) | Minutes after tilt-back | EHBF (*cc./min./1.73 sq. m.*) | Mean arterial pressure (*mm. Hg*) | HPR (*mm. Hg/cc./sec./1.73 sq. m.*) | Pulse rate (*Beats/min.*) |
| Sta | 2 weeks* | 1,336 | 126 | 5.7 | 69 | 3 | 75° | 878 | 79 | 5.4 | 74 | 4 | 969 | 114 | 7.1 | 69 |
| 44 | | 970 | 125 | 7.7 | 69 | 6 | 75° | 780 | 75 | 5.8 | 72 | 12 | 995 | 124 | 7.5 | 66 |
| M | | — | — | — | — | 13 | 55° | 490 | 66 | 8.1 | 72 | 21 | 1,135 | 122 | 6.4 | 66 |
| 1.91 | | | | | | | | | | | | 30 | 1,174 | 123 | 6.3 | 69 |
| Gol | 9½ weeks | 1,005 | 177 | 11.0 | 84 | 5 | 60° | 726 | 143 | 12.0 | 90 | 6 | 1,246 | 178 | 8.6 | 84 |
| 31 | | 1,120 | 184 | 9.9 | 84 | 9 | 60° | 875 | 149 | 10.0 | 84 | 11½ | 1,228 | 177 | 8.6 | 88 |
| M | | 1,381 | 181 | 7.9 | 92 | 15 | 60° | 977 | 147 | 9.0 | 90 | — | — | — | — | — |
| 1.81 | | | | | | | | | | | | | | | | |
| | 7 months | 1,214 | 159 | 7.9 | 70 | 7 | 50° | 1,267 | 150 | 7.1 | 94 | 5½ | 1,956 | 172 | 5.3 | 87 |
| | | 1,366 | 169 | 7.4 | 76 | 15 | 40° | 1,200 | 151 | 7.6 | 90 | 11 | 1,888 | 167 | 5.3 | 82 |
| | | 1,374 | 171 | 7.5 | 74 | 20½ | 40° | 1,276 | 160 | 7.5 | 91 | — | — | — | — | — |
| Pau | 2 weeks | 2,330 | 121 | 3.1 | 72 | 4 | 75° | 1,792† | 71 | 2.4 | ? | 5½ | 1,260 | 127 | 6.0 | 72 |
| 36 | | — | — | — | — | — | — | — | — | — | — | 11½ | 1,678 | 124 | 4.4 | 78 |
| M | | — | — | — | — | — | — | — | — | — | — | 22½ | 1,774 | 127 | 4.3 | 72 |
| 1.84 | | | | | | | | | | | | | | | | |
| Max | 2 weeks | 1,168 | 107 | 5.5 | 60 | 4 | 75° | 899 | 77 | 5.1 | 120 | 5 | 1,496 | 105 | 4.2 | 58 |
| 43 | | 1,435 | 112 | 4.7 | 58 | 7 | 60° | 475‡‖ | 71 | 9.0 | ? | 11 | 1,547 | 109 | 4.2 | 65 |
| M | | — | — | — | — | — | — | — | — | — | — | 20 | 1,300 | 116 | 5.4 | 58 |
| 2.21 | | — | — | — | — | — | — | — | — | — | — | 30½ | 1,255 | 115 | 5.5 | 66 |
| McC | 2 weeks | 3,510 | 158 | 2.7 | 90 | 3½ | 75° | 1,455 | 131 | 5.4 | 120 | 4 | 1,816 | 162 | 5.3 | 96 |
| 43 | | 2,273 | 166 | 4.4 | 96 | 7½ | 75° | 2,434 | 133 | 3.3 | 114 | 11½ | 2,938 | 159 | 3.3 | 90 |
| M | | — | — | — | — | — | — | — | — | — | — | 24 | 1,790 | 159 | 5.3 | 96 |
| 1.75 | | — | — | — | — | — | — | — | — | — | — | 32 | 2,727 | 167 | 3.7 | 96 |
| | 6 months | 1,623 | 177 | 6.5 | 68 | 3½ | 75° | 1,126 | 150 | 8.0 | 96 | 5 | 2,339 | 164 | 4.2 | 64 |
| | | 1,908 | 176 | 5.5 | 68 | 9 | 60° | 974 | 142 | 8.8 | 80 | 14½ | 2,363 | 154 | 3.9 | 72 |
| | | 1,512 | 179 | 7.1 | 68 | 14 | 45° | 1,069 | 144 | 8.1 | 80 | 23 | 1,619 | 158 | 5.9 | 72 |
| | | — | — | — | — | 20 | 45° | 1,352 | 151 | 6.7 | 88 | 32 | 2,029 | 166 | 4.9 | 72 |
| She | 2 weeks | 1,813 | 163 | 5.4 | 84 | 3 | 75° | 2,860† | 104 | 2.2 | 108 | 6½ | 1,535 | 182 | 7.1 | 84 |
| 51 | | — | — | — | — | — | — | — | — | — | — | 11½ | 1,525 | 160 | 6.3 | 88 |
| M | | — | — | — | — | — | — | — | — | — | — | 18 | 1,757 | 162 | 5.5 | 90 |
| 2.02 | | | | | | | | | | | | | | | | |
| | 4 months | 1,568 | 132 | 5.1 | 68 | 9 | 45° | 1,072 | 121 | 6.8 | 84 | 10 | 1,411 | 122 | 5.2 | 72 |
| | | 1,384 | 134 | 5.8 | 68 | 13 | 45° | 831 | 126 | 9.1 | 100 | — | — | — | — | — |
| | | 1,234 | 134 | 6.5 | 68 | 19 | 45° | 939 | 117 | 7.5 | 88 | — | — | — | — | — |
| | | | | | | 25 | 45° | 1,062 | 109 | 6.2 | 84 | — | — | — | — | — |

TABLE I—*Continued*

| Patient Age Sex Surface area (*sq. m.*) | Time after operation | Horizontal position, Control period | | | | Upright position | | | | | | Horizontal position, Recovery period | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | EHBF (*cc./min./ 1.73 sq. m.*) | Mean arterial pressure (*mm. Hg*) | HPR (*mm. Hg/ cc./sec./ 1.73 sq. m.*) | Pulse rate (*Beats/min.*) | Minutes after tilt-up | Degree of tilt | EHBF (*cc./min./ 1.73 sq. m.*) | Mean arterial pressure (*mm. Hg*) | HPR (*mm. Hg/ cc./sec./ 1.73 sq. m.*) | Pulse rate (*Beats/min.*) | Minutes after tilt-back | EHBF (*cc./min./ 1.73 sq. m.*) | Mean arterial pressure (*mm. Hg*) | HPR (*mm. Hg/ cc./sec./ 1.73 sq. m.*) | Pulse rate (*Beats/min.*) |
| Dea<br>54<br>M<br>1.82 | 2 weeks | 1,867 | 159 | 5.1 | 81 | 4 | 75° | 1,328 | 136 | 6.1 | 99 | 3 | 652‖ | 147 | 14.0 | 84 |
| | | 2,286 | 150 | 3.9 | 78 | 7 | 75° | 2,418 | 138 | 3.4 | 102 | 9⅓ | 2,044 | 171 | 5.0 | 87 |
| | | — | — | — | — | — | — | — | — | — | — | 16⅓ | 2,615 | 156 | 3.6 | 84 |
| | 7 months | 1,477 | 176 | 7.1 | 66 | 7 | 75° | 1,113 | 175 | 9.4 | 78 | 11 | 1,569 | 176 | 6.7 | 72 |
| | | 1,545 | 175 | 6.8 | 69 | 11 | 75° | 1,416 | 157 | 6.6 | 79 | 18 | 1,461 | 169 | 6.9 | 66 |
| | | 1,399 | 185 | 7.9 | 72 | 16½ | 75° | 1,270 | 164 | 7.7 | 83 | 23 | 1,275 | 182 | 8.6 | 72 |
| | | — | — | — | — | 21½ | 75° | 1,304 | 165 | 7.6 | 84 | — | — | — | — | — |
| O'Le<br>47<br>F<br>1.56 | 9 years | 1,289 | 126 | 5.9 | 81 | 3½ | 75° | 745† | ? | ? | ? | 7 | 874 | 129 | 8.9 | 82 |
| | | 1,403 | 127 | 5.4 | 80 | — | — | — | — | — | — | 15⅓ | 1,004 | 120 | 7.2 | 75 |
| | | — | — | — | — | — | — | — | — | — | — | 21⅓ | 1,505 | 118 | 4.7 | 75 |
| Lee<br>35<br>M<br>2.21 | 4 years | 779 | 106 | 8.2 | 60 | 20 | 75° | 791 | 108 | 8.2 | 90 | 5⅓ | 839 | 116 | 8.3 | 60 |
| | | 891 | 105 | 7.1 | 52 | 25 | 75° | 746 | 108 | 8.7 | 90 | 13 | 814 | 111 | 8.2 | 68 |
| | | — | — | — | — | — | — | — | — | — | — | 21 | 902 | 111 | 7.4 | 68 |
| Yof<br>42<br>F<br>1.68 | 1 year | 1,152 | 137 | 7.1 | 75 | 6 | 75° | 1,184‡ | ? | ? | ? | 11 | 1,122 | 130 | 7.0 | 75 |
| | | 1,199 | 137 | 6.9 | 75 | — | — | — | — | — | — | 27 | 1,250 | 127 | 6.1 | 74 |
| Bar<br>52<br>F<br>1.85 | 3 years | 943 | 143 | 9.1 | 60 | 4½ | 75° | 780 | 124 | 9.5 | 104 | 5⅓ | 623 | 145 | 14.0 | 51 |
| | | 908 | 149 | 9.8 | 55 | — | — | — | — | — | — | 11⅓ | 736 | 141 | 12.0 | 69 |
| | | 834 | 154 | 11.0 | 71 | — | — | — | — | — | — | 20⅓ | 672 | 146 | 13.0 | 67 |
| Dun<br>30<br>M<br>1.82 | 2 weeks§ | 2,378 | 108 | 2.7 | 84 | 3½ | 75° | 690 | 88 | 7.7 | 155 | 4 | 884 | 130 | 8.8 | 86 |
| | | 2,058 | 108 | 3.2 | 89 | — | — | — | — | — | — | 15½ | 1,218 | 98 | 4.8 | 92 |
| | | 1,687 | 111 | 4.0 | 93 | — | — | — | — | — | — | — | — | — | — | — |

* Patient previously had had an incomplete splanchnicectomy elsewhere; present operation was a total thoracic extension (Rt = D1–D9; Lt = D1–D8) with bilateral excision of greater splanchnic nerves.

† Patient faint, tilted back, sample for EHBF taken immediately.

‡ Patient faint, sample for EHBF taken, tilted back immediately.

‖ This value was obtained during a rapid rise (greater than 0.0006 mg./cc./min.) in the peripheral venous serum concentration of BSP. It is questionable and is deleted from the statistical analysis (Table II).

§ Patient had a supradiaphragmatic splanchnicectomy (Rt = D7–D12; Lt = D8–D12, inclusive).

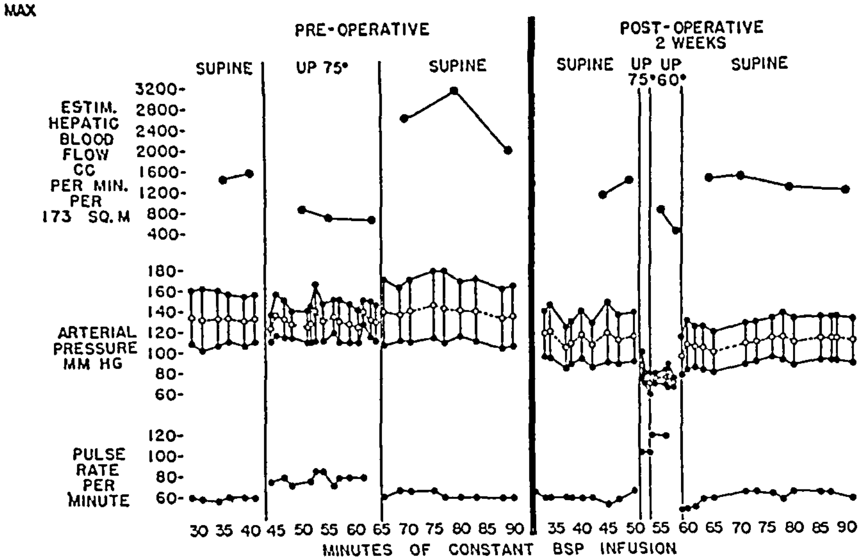

FIG. 1. CHART OF EHBF, ARTERIAL PRESSURE AND PULSE RATE OF A HYPERTENSIVE PATIENT (MAX), STUDIED IN THE HORIZONTAL AND UPRIGHT POSITIONS BEFORE AND AFTER A LUMBODORSAL SPLANCHNICECTOMY

"Mean" (half systolic plus diastolic) arterial pressure is indicated by the open circles and interrupted lines. During and after the upright position in the preoperative test, this patient had a marked anxiety reaction that began with a severe headache.

pertensive patients it increased from 7.3 to 10.1, or 38% (which is highly significant statistically).

The findings in the splanchnicectomized group may also be demonstrated by charts of experiments done on individual cases before and after operation (Figures 1, 2). Here the reductions of EHBF

TABLE II

*The effect of splanchnicectomy on the mean* responses of the estimated hepatic blood flow (EHBF), arterial pressure and hepatic portal resistance (HPR) to upright tilting of the body*

| | EHBF (*cc./min./1.73 sq. m.*) | | | Mean§ arterial pressure (*mm. Hg*) | | | HPR (*mm. Hg/cc./sec./1.73 sq. m.*) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Horizontal (Control) | Upright | Horizontal (Recovery) | Horizontal (Control) | Upright | Horizontal (Recovery) | Horizontal (Control) | Upright | Horizontal (Recovery) |
| Splanchnicectomized patients (11 experiments on 8 patients) | | | | | | | | | |
| Mean | 1,472 | 1,171 | 1,521 | 149 | 125 | 148 | 6.8 | 7.2 | 6.6 |
| Standard Error of Mean | 176 | 163 | 172 | 8 | 9 | 8 | 0.6 | 0.6 | 0.8 |
| Mean Difference† | — | −301 | +360 | — | −24 | +23 | — | +0.4 | −0.5 |
| Significance of Difference (P)‡ | — | <0.01 | <0.01 | — | <0.01 | <0.01 | — | 0.09 | 0.44 |
| Unoperated hypertensive patients (n = 9) | | | | | | | | | |
| Mean | 1,357 | 960 | 1,417 | 158 | 157 | 155 | 7.3 | 10.1 | 7.2 |
| Standard Error of Mean | 97 | 68 | 172 | 7 | 7 | 8 | 0.6 | 0.7 | 0.7 |
| Mean Difference† | — | −397 | +428 | — | −1 | 0 | — | +2.8 | −2.4 |
| Significance of Difference‡ | — . | <0.01 | <0.05 | — | 0.78 | 0.97 | — | <0.01 | <0.01 |

* The mean values of EHBF, arterial pressure and HPR for the group were calculated from the averages of the observations on each individual. See Table I for details of splanchnicectomized patients, and the preceding paper (3) for unoperated hypertensive patients.

† Differences refer to the changes from the immediately preceding position.

‡ The significance of the difference was calculated by the method of Fisher for unique samples (8). P values of 0.05 or less (bold type) denote "significant" differences, and values of 0.01 or less "highly significant" differences.

§ Average of systolic and diastolic pressures.

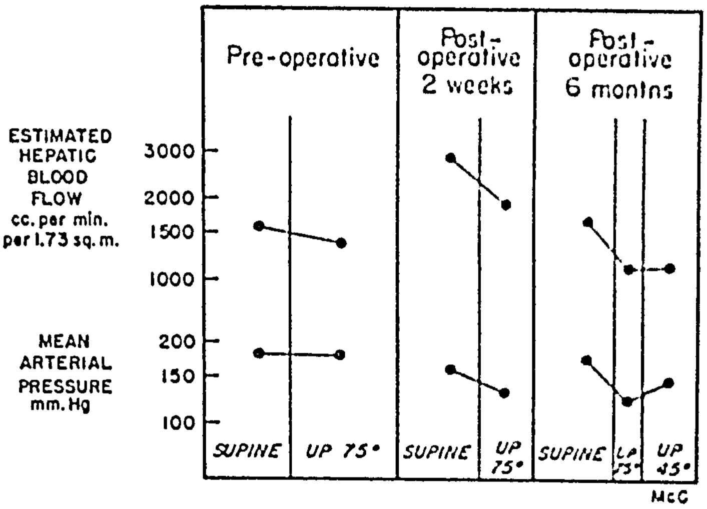

FIG. 2. CHART OF AVERAGE EHBF AND "MEAN" (HALF SYSTOLIC PLUS DIASTOLIC) ARTERIAL PRESSURE IN THE HORIZONTAL AND UPRIGHT POSITIONS IN A HYPERTENSIVE PATIENT (McC) BEFORE, TWO WEEKS AFTER, AND SIX MONTHS AFTER LUMBODORSAL SYMPATHECTOMY

in the upright position before operation are seen to be essentially unrelated to changes in arterial pressure, whereas after operation they were usually associated with sizeable decreases in arterial pressure.

After return to the horizontal position the EHBF in post-operative, as in preoperative, patients usually returned toward, to, or above the previous horizontal control values. Arterial pressure recovered from postural hypotension and calculated HPR did not change significantly (Table II).

## DISCUSSION

The results reported here indicate that the splanchnic sympathetic nervous system of hypertensive patients probably mediates, at least in part, the vasoconstrictor response in the hepatic portal circulation to the upright posture. Thus, sizeable increases in HPR usually did not occur in the upright position after splanchnicectomy, whereas in the same subjects before operation and in normotensive control subjects (3) they did occur. Obviously this does not prove that the changes in EHBF found after splanchnicectomy were only passive and due solely to associated changes in arterial pressure. However, since the decreases in EHBF in the upright position were no greater after than before operation, whereas the reductions in arterial pressure were much greater, one may assume that there was relatively much less active vasoconstriction in the hepatic-portal region after than before operation. These results give direct confirmation to independent hemodynamic studies in this laboratory which have shown that reflex vasopressor responses to certain blood-pressure-lowering stimuli, including the upright posture, are greatly reduced after splanchnicectomy (5, 6).

Besides elucidating the influence of the sympathetic nervous system upon hepatic-portal blood flow, these studies indicate the importance of hydrostatic factors, particularly of orthostatic arterial hypotension, upon EHBF. Decreases in arterial pressure, if sizeable, were usually associated with sizeable reductions of EHBF in sympathectomized patients. On the other hand, when minor changes of arterial pressure such as those usually encountered in normotensive or unsympathectomized hypertensive patients in the upright position occurred in splanchnicectomized patients they were associated with little change in EHBF.

Although for the reasons just given, both the activity of splanchnic sympathetic nervous system and the level of the arterial pressure appear to be important factors in the normal regulation of EHBF, other influences also must be assumed to play a role. Thus, even in well-sympathectomized patients, major "spontaneous" changes in EHBF occurred, particularly during and after orthostasis, that could not be accounted for on the basis of the first two factors alone. For example, after standing upright and being returned to the horizontal position some postoperative patients (Gol, McC, and Dea) had large increases in horizontal EHBF which could not be explained by rises in arterial pressure.

The observations made in patients during orthostatic syncope are also of interest in this connection. As mentioned in a previous paper (3), the tendency to collapse in the upright position before operation seemed usually related directly to the absolute level to which hepatic-portal blood flow fell. Thus, when EHBF fell to 750 cc./min. and failed to recover, collapse was usually imminent even though the arterial pressure up to that point had been well-sustained. After splanchnicectomy, however, while patients in the upright position usually had a lower arterial pressure and a proportionate decrease in EHBF (at times below 750 cc./min.), the expected circulatory "decompensation" characteristic of vaso-vagal syncope did not always appear (notably in Sta). On the other hand, three splanchnicectomized patients (Pau, She and Yof) fainted while EHBF remained good and calculated HPR actually decreased.

The results of these studies provide probably valid indications of the physiologic role of the intact splanchnic sympathetic nervous system, not only in hypertensive but perhaps also in normotensive individuals with respect to the regulation of hepatic-portal blood flow. Along with other observations in this laboratory, they suggest that the sympathetic nervous system normally plays a vasoconstrictor role in the hepatic-portal circulation. The vasoconstrictor and vasodilator influences in this circuit may be presumed usually to be balanced. Conceivably, if they became unbalanced through either overactivity or inhibition of one as compared with the other, blood flow and calculated hepatic-portal resistance would change. Thus, it is possible that the early decrease and late return of increased HPR in resting horizontal hypertensive patients after splanchnicectomy (7) is due to an early unopposed, though normal, vasodilator activity followed by a moderation of that activity or by a return of "intrinsic vasoconstrictor tone," or both. Furthermore, it is possible that the sizeable "spontaneous" variations in EHBF observed after splanchnicectomy may be due to greater or lesser amounts of the remaining "vasodilator activity."

## SUMMARY AND CONCLUSIONS

After splanchnic sympathectomy, reductions of hepatic blood flow in hypertensive patients tilted into the upright position are associated with sizeable decreases in arterial pressure and little change in average hepatic-portal resistance, whereas before operation reductions in EHBF in the upright position occur with little change in pressure and increases in HPR. It is concluded that the splanchnic sympathetic nervous system mediates the hepatic-portal vasoconstrictor response to the upright posture in hypertensive patients and probably also in normal subjects.

## ACKNOWLEDGMENTS

The authors gratefully acknowledge the statistical analysis by Dr. Meyer H. Halperin, the technical assistance of Miss Adele Rymut, and the photographic work of Miss Jane Holbrook.

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# ADRENOCORTICAL CONTROL OF SODIUM AND POTASSIUM EXCRETION IN THE NEWBORN PERIOD[1]

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(Submitted for publication August 14, 1950; accepted, January 2, 1951)

The role of the adrenals in response to disease during the neonatal period has long been a subject of speculation. The anatomical peculiarities of the adrenal cortex in this period are well known. Jaudon (1) has suggested that transient deficiencies in adrenocortical function occur in the newborn period. However, few experimental studies have been reported. Venning (2) has reported that the newborn under one week of age excretes less neutral reducing lipids in response to the administration of adrenocorticotropic hormone (hereinafter called ACTH) than does the older infant. We have found that during the first week of life the decrease in circulating eosinophils after the administration of ACTH is less than in older infants (3).

The author has suggested that stimulation of the newborn adrenal does not cause sodium retention (4). In the present study ACTH was given to newborn infants to study the effect of the adrenal cortex on electrolyte control.

## METHODS

Eleven infants in the premature nursery of the Harriet Lane Home and three full term infants three weeks of age or under and one six months old full term infant on the general ward of the Harriet Lane Home were studied during the winter of 1949–50. The premature infants were all healthy with the exception of two who were being treated for retrolental fibroplasia.[2] The six months old infant was being observed in the hospital for congenital nonhemolytic jaundice. Two of the other three patients on the infants ward had recovered from the acute pneumonitis for which they were admitted. The third patient had congenital glaucoma.

Complete urine collections were made with the few exceptions to be noted in the tables. Soft rubber tubing fitting snugly over the penis was used for collecting urine from the males. Urine was collected from female patients by placing the infant in an ordinary bassinet with the head of the bassinet slightly elevated, and with the patient's buttocks over a Buchner funnel. The patients were supported in this position by slings supporting their lower thighs. Five cc. of 25% acetic acid were added to each day's urine collection.

Patients were weighed routinely three times a week on the regular balances of the wards and nursery. Weights are recorded for the beginning of the daily periods.

Eosinophil counts were made by the method of Randolph (5). Sodium and potassium in the urine were measured by an internally compensated Perkin-Elmer flame photometer. 17-Ketosteroids and neutral reducing lipids were measured by methods previously described (6). Nitrogen in the urine was determined by nesslerization. Serum and stool electrolytes were not determined. Dietary intake was calculated from conventional tables and random samples were analyzed for sodium and potassium content using the wet ashing method of Wallace (7) and measuring with a flame photometer. The volume of feeding mixture in each bottle was accurately measured, but when any of the bottle was refused, the amount was estimated by the nurse using the calibrations on the bottle. Fortunately, this occurred rarely.

ACTH[3] was given to 11 patients for one day only, one receiving 10 mg. intramuscularly every six hours, one 10 mg. every eight hours, six 5 mg. every six hours, two 2 mg. every six hours, and one 1 mg. every six hours. Two other patients received ACTH four times daily for periods of seven and 10 days respectively.

When desoxycorticosterone acetate (hereinafter called DCA) or cortisone were used they were given as a single intramuscular injection at the beginning of the daily period.

## RESULTS

Seven of the newborn infants tested with ACTH for one day were prematures who had weighed between 1,210 and 1,800 grams at birth (Cases 1–4, 10, 11, 14). At the time of the study their weights were between 1,330 and 3,125 grams.

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[1] This work was made possible by a grant from the American Cancer Society for studies on the relationship of the pituitary hormones, thyroid hormone, and the steroid hormones of the adrenal glands and gonads to normal and abnormal growth, and by a grant from the Commonwealth Fund for the study of endocrine problems in childhood.

[2] These were treated under the supervision of Drs. W. and E. Owens.

[3] Kindly supplied by Dr. J. R. Mote of the Armour Laboratories, Chicago, Illinois.

The oldest was 41 days old. In addition three full term infants three weeks of age or less and weighing between 3,150 and 4,150 grams were studied. Sodium retention on the day of ACTH administration in these cases was minimal or non-existent. However, in all but one of the patients there was a significant increase in excretion of sodium in the urine on the day following administration of ACTH. This loss in the urine was often greater than the calculated intake for the day. It was accompanied by an increase in urinary volume. There was usually a decreased rate of the weight gain in this period. The one infant who did not show this sodium loss was one of those receiving the smallest doses of ACTH.

The effect of ACTH on potassium excretion was more variable. In some infants (and these usually were among the larger who received the larger doses) there was an increased urinary excretion of potassium on the same day that ACTH was given whereas there was no change in urinary sodium until the following day. In other infants, potassium excretion did not change or else varied directly with the volume of urine. The initial rise in potassium excretion was invariably associated with increased urinary nitrogen excretion. Usually the amount of nitrogen excretion did not indicate that there was sufficient protein catabolism to account for the potassium lost. In several cases there was a secondary rise in potassium excretion two or three days after ACTH was given. This, too, was always associated with a secondary rise in nitrogen excretion.

The nitrogen excretion in the urine showed a uniform rise when ACTH was administered. This occurred either on the day ACTH was given or on the following day.

In all those infants over one week of age on whom counts were made, there was a fall in circulating eosinophils irrespective of the dose of ACTH. Since these results were in accord with previous work (3), such counts were made only in the first few studies. It is of importance to note that there was the expected rise in excretion of 17-ketosteroids and neutral reducing lipids after administration of ACTH. On continued stimulation, the 17-ketosteroid levels rose at times to normal adult female levels and the neutral reducing lipids far exceeded normal adult levels.

Since in these newborns ACTH failed to cause sodium retention which usually occurs in older children and adults, it is important to note the effect of DCA in this group of patients. The two newborns who were given 5 mg. of DCA in a single intramuscular injection and the one who was given 2 mg. showed a definite decrease in urine volume and sodium excretion (Cases 9–11). The patient who received only 1 mg. of DCA showed no definite sodium retention although his urine volume did decrease. DCA caused no increase in potassium excretion, but instead, in some cases, there was a decrease parallel to the reduction in urine volume.

Two premature infants were given cortisone (Cases 13, 14). When 50 mg. were given to the first infant there was no significant change in electrolyte excretion. The second infant who received 25 mg. of cortisone showed marked urinary retentions of water, sodium and potassium. This

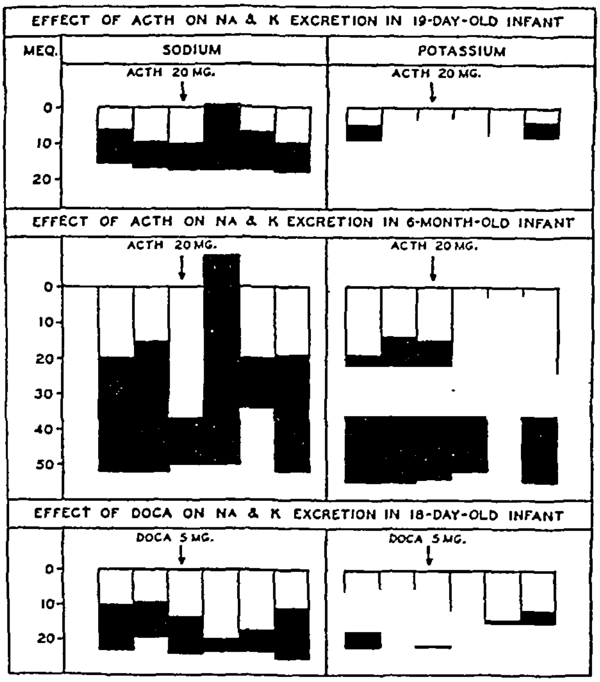

FIG. 1. ELECTROLYTE BALANCES OF A 19 DAY OLD INFANT GIVEN ACTH, AND OF A SIX MONTH OLD INFANT GIVEN ACTH AND AN 18 DAY OLD INFANT GIVEN DCA

Each column represents one day. Intakes are plotted down from the zero line, and urinary excretion (blacked in) is plotted up from the intake. The resultant white spaces below the zero line represent positive balances and the blacked in areas above the zero line represent negative balances. No allowance is made for stool or sweat electrolytes.

infant then was given ACTH and responded to it in the same manner as the other newborns.

In addition to the ten infants who were treated with ACTH for only 24 hours, two premature infants were treated with ACTH for seven and 10 days respectively in an experimental attempt to influence retrolental fibroplasia (Cases 7, 8). Neither of these patients showed any signs of escape from the effects of ACTH on electrolyte balance. The increased sodium excretion continued for two or three days after discontinuing ACTH. One of these prematures developed diarrhea while being treated with ACTH (Case 8). He continued to pour out more sodium in spite of the fact that there was probably an increased loss of sodium in the stools. His weight fell sharply and it was felt that the magnitude and rapidity of the loss were greater than would be expected from the relative mildness of his diarrhea. Three days after ACTH was discontinued conservation of sodium occurred due to a decrease in the urinary excretion. Until this time the additional amounts of parenteral saline received were excreted within 24 hours in the urine. He was able to retain potassium better even while getting ACTH, although one day while he was having diarrhea the urinary excretion exceeded the intake. The urinary volume remained high until one week after

TABLE I

*Electrolyte and steroid data for 15 infants studied*

| Case | Day | Rx | Wt. | Intake |  |  | Urinary output |  |  |  | Urinary steroids |  |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
|  |  |  |  | Vol. | Na | K | Vol. | Na | K | N | 17KS | Neutral red. lipids |
|  |  |  | *grams* | *cc.* | *meq* | *meq* | *cc.* | *meq* | *meq* | *grams* | *mg.* | *mg.* |
| Case 1<br>18 days<br>birth wt.<br>1,765 grams | 1 | 0 | 1,600 | 82 | 5.5 | 4.6 | 84 | 4.5 | 3.8 | 0.29 | 0.54 | 0.34 |
|  | 2 | 0 |  | 205 | 13.9 | 11.5 | 72 | 4.8 | 4.2 | 0.34 | 0.44 | 0.31 |
|  | 3 | ACTH 4 mg. | 1,660 | 210 | 14.3 | 11.7 | 70 | 4.8 | 5.5 | 0.38 | 0.94 | 0.57 |
|  | 4 | 0 |  | 210 | 14.3 | 11.7 | 65 | 5.2 | 3.5 | 0.32 | 0.54 | 0.56 |
|  | 5 | 0 | 1,775 | 210 | 14.3 | 11.7 | 54 | 3.1 | 3.1 | 0.17 | 0.32 | — |
| Case 1<br>at 33 days | 1 | 0 |  | 430 | 18.0 | 16.9 | incomplete collection |  |  |  |  |  |
|  | 2 | 0 | 2,400 | 510 | 18.0 | 16.9 | 240 | 6.7 | 6.5 | 0.87 | 0.85 | 0.29 |
|  | 3 | ACTH 4 mg. |  | 510 | 18.0 | 16.9 | 240 | 8.6 | 8.9 | 1.45 | 1.43 | 0.28 |
|  | 4 | 0 | 2,465 | 450 | 18.0 | 16.9 | 203 | 11.8 | 9.1 | 0.13 | 1.22 | 0.28 |
|  | 5 | 0 |  | 510 | 18.0 | 16.9 | 194 | 7.4 | 7.4 | 0.08 | 0.86 | 0.26 |
|  | 6 | 0 | 2,600 | 485 | 18.0 | 16.9 | 238 | 8.1 | 8.8 | 0.67 | 0.97 | 0.29 |
| Case 2<br>9 days<br>birth wt.<br>1,430 grams | 1 | 0 | 1,330 |  |  |  |  |  |  |  |  |  |
|  | 2 | 0 |  | 126 | 8.3 | 7.0 | 60 | 3.1 | 3.0 | 0.27 | 0.01 | 0.06 |
|  | 3 | 0 | 1,345 | 146 | 9.6 | 8.0 | 46 | 3.2 | 4.3 | 0.31 | 0.00 | 0.06 |
|  | 4 | 0 |  | 150 | 9.9 | 8.3 | 50 | 2.5 | 4.3 | 0.26 | 0.04 | 0.05 |
|  | 5 | ACTH 8 mg. | 1,430 | 175 | 11.5 | 9.6 | 60 | 3.3 | 5.4 | 0.30 | 0.50 | 0.12 |
|  | 6 | 0 |  | 180 | 11.9 | 9.9 | 110 | 11.2 | 4.1 | 0.41 | 0.39 | 0.14 |
|  | 7 | 0 |  | 180 | 11.9 | 9.9 | 84 | 7.2 | 5.6 | 0.31 | 0.00 | 0.11 |
|  | 8 | 0 | 1,495 | 205 | 13.5 | 11.5 | 33 | 2.8 | 6.3 | 0.38 | 0.37 | 0.08 |
| Case 2<br>at 41 days | 1 | 0 | 2,125 | 375 | 15.0 | 13.9 | 188 | 7.1 | 11.1 | 0.99 | 0.56 | 0.10 |
|  | 2 | 0 | 2,235 | 345 | 13.8 | 12.8 | 152 | 6.4 | 9.9 | 0.97 | 0.58 | 0.12 |
|  | 3 | ACTH 8 mg. |  | 370 | 14.8 | 13.7 | 218 | 11.1 | 12.6 | 1.22 | 0.87 | 0.22 |
|  | 4 | 0 |  | 390 | 15.6 | 14.4 | 200 | 13.6 | 9.8 | 1.64 | 0.80 | 0.14 |
|  | 5 | 0 | 2,340 | 360 | 14.4 | 13.4 | 160 | 7.0 | 13.0 | 0.94 | 0.90 | 0.13 |
|  | 6 | 0 |  | 390 | 15.6 | 14.4 | 150 | 6.6 | 9.0 | 0.88 | 0.90 | 0.12 |
|  | 7 | 0 | 2,450 | 390 | 15.6 | 14.4 | 170 | 8.3 | 13.1 | 0.91 | 0.62 | — |
| Case 3<br>11 days<br>birth wt.<br>2,100 grams | 1 | 0 | 2,200 | 350 | 13.5 | 13.0 | 185 | 6.1 | 4.1 |  | 0.76 | 0.21 |
|  | 2 | 0 |  | 345 | 13.8 | 12.8 | 170 | 5.4 | 3.9 |  | 0.60 | 0.18 |
|  | 3 | ACTH 8 mg. | 2,500? | 350 | 14.0 | 13.0 | 142+ | 5.7 | 4.8 |  | 1.00 | 0.25 |
|  | 4 | 0 |  | 355 | 14.2 | 13.2 | 155 | 5.4 | 3.4 |  | 0.88 | 0.17 |
|  | 5 | 0 | 2,450 | 375 | 15.0 | 13.9 | 165 | 6.9 | 5.1 |  | 0.92 | 0.19 |
| Case 4<br>19 days<br>birth wt.<br>1,800 grams | 1 | 0 | 2,320 | 390 | 15.6 | 14.4 | 150 | 9.5 | 9.7 | 0.55 | 0000 | — |
|  | 2 | 0 |  | 415 | 16.6 | 15.4 | 115 | 6.9 | 6.7 | 0.49 | 0000 | 0.18 |
|  | 3 | ACTH 20 mg. | 2,400 | 420 | 16.8 | 15.6 | 150 | 6.4 | 12.3 | 1.03 | 1.20 | 0.23 |
|  | 4 | 0 |  | 420 | 16.8 | 15.6 | 195 | 17.0 | 7.8 | 0.97 | 0.72 | — |
|  | 5 | 0 |  | 420 | 16.8 | 15.6 | 130 | 10.0 | 7.5 | 0.72 | 0000 | — |
|  | 6 | 0 | 2,455 | 445 | 17.8 | 16.5 | 175 | 7.7 | 12.1 | 1.02 | 0000 | 0.14 |

TABLE I—*Continued*

| Case | Day | Rx | Wt. | Intake | | | Urinary output | | | | Urinary steroids | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Vol. | Na | K | Vol. | Na | K | N | 17KS | Neutral red. lipids |
| | | | *grams* | *cc.* | *meq* | *meq* | *cc.* | *meq* | *meq* | *grams* | *mg.* | *mg.* |
| Case 5 | 1 | 0 | 3,150 | 550 | 22.0 | 20.6 | 170 | 11.8 | 14.6 | 0.83 | 0.80 | |
| 21 days | 2 | 0 | 3,140 | 580 | 23.3 | 21.8 | 195 | 13.2 | 15.4 | 0.87 | 1.10 | |
| birth wt. | 3 | ACTH 20 mg. | | 555 | 22.3 | 20.8 | 205 | 8.8 | 18.0 | 1.05 | 2.40 | |
| 2,800 grams | 4 | 0 | 3,220 | 600 | 24.1 | 22.5 | 295 | 30.0 | 13.8 | 1.67 | 1.80 | |
| | 5 | 0 | | 590 | 23.7 | 22.1 | 265 | 20.6 | 18.3 | 1.14 | 0.74 | |
| | 6 | 0 | 3,460 | 530 | 21.3 | 19.4 | 150 | 14.1 | 25.8 | 1.78 | 0.66 | |
| Case 6 | 1 | 0 | 3,500 | 360 | 14.4 | 13.3 | 45 | 3.6 | 3.6 | | | |
| 17 days | 2 | 0 | | 585 | 20.4 | 21.5 | 107 | 6.6 | 7.7 | | | |
| birth wt. | 3 | ACTH 20 mg. | 3,500 | 580 | 22.6 | 24.8 | 160 | 6.9 | 18.8 | | | |
| 3,209 grams | 4 | 0 | 3,500 | 605 | 22.6 | 22.5 | 185 | 13.9 | 9.6 | | | |
| | 5 | 0 | | 600 | 17.6 | 18.6 | 270 | 11.6 | 16.2 | | | |
| Case 7 | 1 | 0 | 3,125 | 600 | 24.0 | 22.4 | 110 | 5.4 | 6.4 | 0.39 | 0.90 | 0.30 |
| 69 days | 2 | ACTH 20 mg. | | 600 | 24.0 | 22.4 | 200 | 6.2 | 15.6 | 0.97 | 0.90 | 0.35 |
| birth wt. | 3 | 20 | | 600 | 24.0 | 22.4 | 320 | 14.7 | 19.6 | 1.73 | — | — |
| 1,600 grams | 4 | 20 | 3,225 | 600 | 24.0 | 22.4 | 340 | 14.6 | 16.0 | 2.74 | 5.50 | — |
| | 5 | 20 | | 600 | 24.0 | 22.4 | incomplete collection, concentrations of same order | | | | | |
| | 6 | 20 | 3,385 | 600 | 24.0 | 22.4 | of magnitude as the previous days for both days | | | | | |
| | 7 | ACTH 40 mg. | | 600 | 24.0 | 22.4 | 240 | 22.6 | 15.1 | 1.82 | — | — |
| | 8 | 40 | 3,480 | 600 | 26.9 | 23.4 | 325 | 32.5 | 12.7 | — | 7.20 | 2.16 |
| | 9 | 0 | | 585 | 26.9 | 22.4 | 240 | 33.0 | 7.2 | 1.56 | — | — |
| | 10 | 0 | | 600 | 28.0 | 25.6 | 350 | 37.8 | 16.0 | 1.04 | 2.00 | — |
| | 11 | 0 | 3,270 | 600 | 28.0 | 25.6 | 150 | 5.0 | 15.0 | — | — | — |
| | 12 | 0 | | 600 | 28.0 | 25.6 | 140 | 11.2 | 9.5 | 1.14 | 0.90 | — |
| | 13 | 0 | 3,500 | | | | | | | | | |
| Case 8 | 1 | 0 | 2,155 | 360 | 14.4 | 13.3 | 105 | 6.5 | 6.3 | 0.68 | — | — |
| 52 days | 2 | 0 | 2,205 | 360 | 14.4 | 13.3 | 155 | 7.8 | 6.3 | 0.80 | 1.60 | — |
| birth wt. | 3 | 0 | | 360 | 14.4 | 13.3 | 115 | 5.2 | 5.4 | 0.59 | 1.64 | 0.11 |
| 1,285 grams | 4 | ACTH 8 mg. | 2,295 | 355 | 14.2 | 13.2 | 110 | 5.8 | 5.8 | 0.71 | 1.66 | — |
| | 5 | ACTH 10 mg. | | 360 | 14.4 | 13.3 | 125 | 7.5 | 8.6 | 0.51 | — | — |
| | 6 | 10 | | 380 | 15.2 | 14.1 | 158 | 9.5 | 5.9 | 0.95 | 2.40 | — |
| | 7 | ACTH 20 mg. | 2,220 | 440 | 17.6 | 16.3 | no collection | | | | | |
| | 8 | 20 | | 450 | 18.0 | 16.7 | 210 | 17.4 | 6.9 | 2.10 | 5.80 | 0.50 |
| | 9 | 20 | 2,260 | 450 | 18.0 | 16.7 | no collection | | | | | |
| | 10 | 20 | | 400 | 16.0 | 14.8 | 195 | 17.0 | 7.6 | 2.10 | 3.20 | |
| | 11 | 20 | 2,180 | 325 | 13.0 | 12.0 | no collection | | | | | |
| | 12 | 20 | 2,125 | 300 | 00 | 00 | 205 | 9.0 | 5.1 | 1.80 | — | — |
| | 13 | 20 | 2,045 | 400 | 3.0 | 00 | no collection | | | | | |
| | 14 | 0 | 1,945 | 425 | 21.8 | 9.1 | 185 | 7.9 | 1.0 | 1.00 | — | — |
| | 15 | 0 | 2,040 | 390 | 8.1 | 11.7 | 290 | 24.5 | 2.7 | 1.10 | — | — |
| | 16 | 0 | 2,090 | 440 | 10.9 | 13.8 | 188 | 0.6 | 6.0 | 0.71 | 0.50 | — |
| | 17 | 0 | 2,060 | 440 | 11.6 | 13.6 | no collection | | | | | |
| | 18 | 0 | 2,160 | 390 | 8.9 | 14.0 | 165 | 0.3 | 7.4 | 1.20 | — | — |
| | 19 | 0 | 2,280 | 390 | 9.2 | 14.6 | no collection | | | | | |
| | 20 | 0 | 2,360 | 390 | 11.9 | 17.8 | 88 | 2.2 | 6.5 | 0.29 | — | — |
| | 21 | 0 | 2,470 | | | | | | | | | |
| Case 9 | 1 | 0 | 4,150 | 575 | 23.0 | 21.6 | 177 | 12.9 | 15.8 | — | | |
| 18 days | 2 | 0 | 4,170 | 465 | 18.8 | 17.5 | 118 | 9.8 | 12.3 | | | |
| birth wt. | 3 | DCA 5 mg. | | 595 | 23.8 | 22.4 | 128 | 10.4 | 10.6 | | | |
| 3,800 grams | 4 | 0 | 4,200 | 590 | 23.6 | 22.2 | 90 | 3.4 | 7.2 | | | |
| | 5 | 0 | | 590 | 23.6 | 22.2 | 137 | 6.6 | 7.8 | 0.82 | 0.40 | — |
| | 6 | 0 | | 625 | 25.0 | 23.5 | 215 | 13.5 | 12.4 | 1.36 | 0.50 | 0.18 |
| | 7 | ACTH 40 mg. | | 685 | 27.3 | 25.6 | 240 | 10.8 | 18.0 | 1.75 | 0.60 | 0.48 |
| | 8 | 0 | 4,520 | 680 | 27.1 | 25.3 | 292 | 28.8 | 12.0 | 1.39 | 0.67 | 0.15 |
| | 9 | 0 | 4,510 | 670 | 26.8 | 25.1 | 290 | 13.9 | 21.2 | 2.21 | 0.60 | — |
| | 10 | 0 | 4,610 | 710 | 28.4 | 26.6 | 270 | 17.0 | 23.2 | 1.79 | 0.73 | — |

TABLE 1—*Continued*

| Case | Day | Rx | Wt. | Intake |  |  | Urinary output |  |  |  | Urinary steroids |  |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
|  |  |  |  | Vol. | Na | K | Vol. | Na | K | N | 17KS | Neutral red. lipids |
|  |  |  | *grams* | *cc.* | *meq* | *meq* | *cc.* | *meq* | *meq* | *grams* | *mg.* | *mg.* |
| Case 10 | 1 | 0 | 1,560 | 210 | 10.9 | 8.2 | 60+ | 5.4 | 3.3 |  |  |  |
| 21 days | 2 | 0 |  | 210 | 10.9 | 8.2 | 96 | 7.2 | 4.3 |  |  |  |
| birth wt. | 3 | DCA 5 mg. | 1,615 | 220 | 11.1 | 8.6 | 53 | 3.4 | 2.9 |  |  |  |
| 1,210 grams | 4 | 0 |  | 226 | 11.8 | 8.8 | 50 | 2.8 | 3.2 |  |  |  |
|  | 5 | 0 |  | 238 | 12.4 | 9.3 | 50 | 3.3 | 2.9 |  |  |  |
|  | 6 | 0 | 1,760 | 240 | 12.5 | 9.4 | 65 | 4.8 | 2.6 |  | 0.33 |  |
|  | 7 | 0 |  | 240 | 12.5 | 9.4 | 90 | 9.0 | 4.4 |  | 0.20 |  |
|  | 8 | ACTH 20 mg. | 1,850 | 260 | 13.5 | 10.1 | 60 | 4.4 | 6.1 |  | 0.73 |  |
|  | 9 | 0 |  | 270 | 14.1 | 10.8 | 150+ | 18.3 | 2.7 |  | 0.60 |  |
|  | 10 | 0 | 1,860 | 295 | 15.4 | 11.8 | 100 | 4.7 | 7.0 |  | — |  |
|  | 11 | 0 | 2,050 |  |  |  |  |  |  |  |  |  |
| Case 11 | 1 | 0 | 1,460 | 210 | 13.8 | 11.5 | 100 | 7.0 | 6.0 | 0.62 | 0.70 | — |
| 20 days | 2 | 0 |  | 218 | 14.2 | 12.0 | 110 | 8.2 | 8.5 | 0.66 | 0.60 | 0.15 |
| birth wt. | 3 | ACTH 20 mg. | 1,560 | 230 | 15.2 | 12.6 | 115 | 7.4 | 8.0 | 0.73 | 0.60 | 0.26 |
| 1,250 grams | 4 | 0 |  | 240 | 15.8 | 13.2 | 160 | 17.6 | 7.0 | 1.12 | 1.20 | 0.41 |
|  | 5 | 0 |  | 250 | 16.4 | 13.7 | 98 | 8.1 | 5.7 | 0.62 | 0.40 | — |
|  | 6 | 0 | 1,565 | 267 | 17.6 | 14.7 | 160 | 4.7 | 8.4 | 0.50 | 0.40 | 0.09 |
|  | 7 | DCA 2 mg. |  | 270 | 17.8 | 14.8 | 125 | 8.0 | 9.0 | 0.75 |  |  |
|  | 8 | 0 | 1,655 | 270 | 17.8 | 14.8 | 73 | 5.9 | 9.0 |  |  |  |
|  | 9 | 0 |  | 270 | 17.8 | 14.8 | 68 | 7.3 | 5.2 |  |  |  |
|  | 10 | 0 | 1,715 | 270 | 17.8 | 14.8 | 78 | 8.4 | 5.8 |  |  |  |
| Case 12 | 1 | 0 | 2,130 |  |  |  |  |  |  |  |  |  |
| 18 days | 2 | 0 |  | 270 | 10.8 | 10.0 | 138 | 8.0 | 6.3 |  |  |  |
| birth wt. | 3 | 0 | 2,195 | 320 | 12.8 | 11.9 | 145 | 8.2 | 9.4 |  |  |  |
| 1,630 grams | 4 | 0 |  | 330 | 13.2 | 12.2 | 165 | 9.0 | 9.4 |  |  |  |
|  | 5 | DCA 1 mg. | 2,325 | 320 | 12.8 | 11.9 | 100 | 7.2 | 6.8 |  |  |  |
|  | 6 | 0 |  | 325 | 13.0 | 12.0 | 164 | 11.0 | 9.0 |  |  |  |
|  | 7 | 0 |  | 330 | 13.2 | 12.2 | 150 | 8.4 | 8.7 |  |  |  |
| Case 13 | 1 | 0 |  | 265 | 13.9 | 10.5 | 84 | 6.0 | 5.6 | 0.27 |  |  |
| 22 days | 2 | 0 | 1,860 | 280 | 14.7 | 11.0 | 85 | 5.5 | 5.3 | 0.35 | 0.85 | — |
| birth wt. | 3 | 0 |  | 295 | 15.5 | 11.6 | 106 | 7.6 | 6.3 | 0.34 | 1.06 | 0.14 |
| 1,540 grams | 4 | Cortisone 50 mg. | 1,940 | 290 | 15.3 | 11.4 | 93 | 7.0 | 6.3 | 0.36 | 1.14 | 0.13 |
|  | 5 | 0 |  | 300 | 15.8 | 11.9 | 130 | 7.8 | 7.3 | 0.50 | 1.22 | 0.18 |
|  | 6 | 0 |  | 300 | 15.8 | 11.9 | 155 | 9.9 | 7.4 | 0.49 | 0.93 | — |
|  | 7 | 0 | 2,005 | 300 | 15.8 | 11.9 | 84 | 5.7 | 5.1 | 0.38 | 0.16 | 0.09 |
|  | 8 | 0 |  | 295 | 15.5 | 11.6 | 118 | 7.3 | 6.7 | 0.46 | 0.06 | — |
|  | 9 | 0 | 2,075 |  |  |  |  |  |  |  |  |  |
| Case 14 | 1 | 0 | 1,650 | 240 | 16.3 | 13.4 | 116 | 8.9 | 9.6 | 0.54 | 0.77 |  |
| 25 days | 2 | 0 |  | 240 | 16.3 | 13.4 | 110 | 7.5 | 8.3 | 0.46 | 0.70 | 0.08 |
| birth wt. | 3 | Cortisone 25 mg. | 1,735 | 240 | 16.3 | 13.4 | 40 | 2.9 | 3.0 | 0.22 | 3 day aver. | 2 day aver. |
| 1,455 grams | 4 | 0 |  | 240 | 16.3 | 13.4 | 70 | 2.9 | 2.7 | 0.25 | 0.71 | 0.05 |
|  | 5 | 0 |  | 265 | 18.0 | 14.8 | 73 | 3.5 | 4.5 | 0.26 |  |  |
|  | 6 | 0 | 1,825 | 270 | 18.3 | 15.1 | 111 | 7.5 | 7.2 | 0.44 | 0.84 | 2 day aver. |
|  | 7 | 0 |  | 270 | 18.3 | 15.1 | 85 | 6.0 | 6.1 | 0.38 | 0.58 | 0.13 |
|  | 8 | ACTH 30 mg. | 1,950 | 280 | 19.0 | 15.7 | 117 | 6.1 | 9.7 | 0.57 | 0.73 | 0.15 |
|  | 9 | 0 |  | 300 | 20.4 | 16.8 | 120+ | 12.5 | 6.6 | 0.75 | 0.74 | 0.19 |
|  | 10 | 0 | 1,965 | 325 | 22.0 | 18.2 | 120 | 12.4 | 7.0 | 0.53 | 0.64 | — |
|  | 11 | 0 |  | 330 | 22.5 | 18.5 | 95 | 4.3 | 10.5 | 0.89 | 0.51 | 0.11 |
|  | 12 | 0 |  | 330 | 22.5 | 18.5 | 117 | 9.1 | 10.3 | 0.78 | 0.63 | — |
|  | 13 | 0 | 2,025 | 330 | 22.5 | 18.5 | 133 | 11.0 | 10.9 | 0.80 | 0.77 | — |
|  | 14 | 0 | 2,105 |  |  |  |  |  |  |  |  |  |
| Case 15 | 1 | 0 | 7,020 | 1,025 | 52.0 | 55.0 | 370 | 32.0 | 36.0 |  | 0.70 |  |
| 6 months | 2 | 0 |  | 1,025 | 52.0 | 55.0 | 415 | 37.0 | 41.0 |  | 0.50 |  |
| birth wt. | 3 | ACTH 20 mg. |  | 1,025 | 50.0 | 54.0 | 260 | 13.0 | 39.0 |  | 0.70 |  |
| 3,500 grams | 4 | 0 | 7,100 | 960 | 50.0 | 52.0 | 535 | 59.0 | 30.0 |  | 0.90 |  |
|  | 5 | 0 |  | 730 | 34.0 | 36.0 | 235 | 14.0 | 33.0 |  | 0.50 |  |
|  | 6 | 0 |  | 1,000 | 52.0 | 55.0 | 225 | 33.0 | 31.0 |  | 0.50 |  |

ACTH was discontinued. Sodium concentration in the urine never exceeded 87 meq/l.

In comparison with the infants of the younger age group a study was made on one six month old full term infant (Case 15). He showed sodium retention when given ACTH for one day in a manner entirely similar to that shown by the usual adult receiving ACTH for one day.

Figures for the intake and excretion of ions and for the excretion of steroids and nitrogen in these cases are given in Table I. Figure 1 compares the electrolyte balance of a characteristic newborn given ACTH and also DCA with that of a six month old infant given ACTH.

## DISCUSSION

The functional immaturity of the kidney of the infant is well known having been elucidated by McCance and Young (8), Rubin, Bruck and Rapoport (9), and others. However, it is difficult to account for this peculiar electrolyte response to ACTH on this basis since the administration of DCA to the newborn caused decreased excretion of sodium as it does in the older child and adult. This suggests that the kidney of the newborn is capable of responding normally to adrenocortical hormone. It seems more probable that the peculiarity of electrolyte excretion is due to a difference in the hormones secreted by the immature adrenal. This might be due either to the secretion of a hormone not present in later life or to an alteration in the balance between hormones having varying quantitative or qualitative effects on sodium excretion. The latter alternative might be considered on the basis of the theory advanced by Thorn and associates (10) suggesting that weak sodium retainers such as Compound E and F compete with potent sodium retainers such as DCA and thereby induce salt loss. Thus it is possible that in the case of the newborn the adrenal when stimulated by ACTH may secrete relatively more Compound F-like hormone than the adult adrenal under similar circumstances. In patients with congenital hyperplasia the ACTH-induced secretion of androgenic steroids might also compete with DCA-like steroids, and thereby induce the observed sodium loss (6, 11).

In our small series there was no demonstrable difference in response between premature and full term infants of the same post-natal age, but it is possible that the abnormal effects of the adrenal on electrolyte excretion persist longer in premature than in full term infants.

## SUMMARY AND CONCLUSIONS

Twelve newborn infants were given ACTH for periods of one day to ten days. This was regularly accompanied by the expected increase in the excretion of neutral reducing lipid, 17-ketosteroids, and urinary nitrogen. The urinary excretion of potassium showed only moderate changes, usually increasing on the day of ACTH injection as expected from adult experience. Sodium excretion was always increased one day after an adequate dose of ACTH was given. This frequently was of sufficient magnitude to exceed the dietary intake of this ion. One six month old infant retained sodium after the administration of ACTH in the same manner as adults. When DCA was administered to newborns they reacted like adults, showing a decreased excretion of sodium. In one newborn, 25 mg. of cortisone produced sodium retention; 50 mg. of cortisone given to another newborn produced no effect on the sodium balance.

## ACKNOWLEDGMENTS

The technical assistance of Mr. Henry Schulte and Mrs. Mary Ellen Crafton in carrying out the steroid and nitrogen determinations is gratefully acknowledged.

The author wishes to thank Dr. Lawson Wilkins for his advice and encouragement in the carrying out of the study and for his help in the preparation of the manuscript.

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# STUDIES ON THE COMBINED USE OF MEASUREMENTS OF SWEAT ELECTROLYTE COMPOSITION AND RATE OF SWEATING AS AN INDEX OF ADRENAL CORTICAL ACTIVITY[1]

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(Submitted for publication October 25, 1950; accepted, January 2, 1951)

## INTRODUCTION

Since Conn and his associates (1, 2) proposed that the concentration of chloride or sodium in thermal sweat can be used as an index of that function of the adrenal cortex concerned primarily with electrolyte metabolism, studies concerning the factors which are related to or influence the electrolytic composition of sweat have gained greatly in significance. In the past, several investigators have obtained data which suggested that the concentration of chloride in sweat is positively correlated with the rate of sweating (3, 4) and skin temperature (5). However, the relations of these variables to sweat electrolyte composition have never been defined with precision nor have they been studied in connection with the effect of adrenal cortical activity on the composition of sweat.

The present paper is concerned initially with studies which show that certain non-endocrine factors play a major role in the determination of sweat electrolyte composition. A method for taking these factors into consideration in the utilization of sweat electrolyte measurements as an index of hormonal activity is described. Finally investigations of adrenal cortical function based on this newly developed index are presented.

## METHODS

Unless otherwise noted subjects were maintained on a standard diet for three to five days prior to the first collection of sweat and throughout the interval during which the collections were made. The *standard* daily diet consisted of a low-salt diet, estimated to contain 1.5 gms. of sodium chloride, supplemented with weighed sodium chloride in such amounts as to bring the total sodium chloride intake up to 5 gms. per square meter of body surface ($m^2$) per day. Drinking water was allowed *ad lib.*

Sweating was induced by placing the subject in a room the temperature of which could be maintained at the desired level by automatic temperature regulators. Usually additional stimulation was provided by having the subject immerse one arm in a bath of warm water, the temperature of which was kept nearly constant at the value desired throughout the collection period.

Sweat was collected from the non-immersed arm by enclosing it in a loosely-fitting watertight envelope, made of rubber dental dam and fitted with a glass delivery tube at its lower end. Immediately before the envelope was placed in position, the skin of the arm was thoroughly cleaned with tap water, 70 per cent aqueous solution of ethanol and finally, distilled water.

Rates of sweating were expressed as ml. per hour per 0.1 $m^2$ of the skin surface enclosed in the envelope, the surface area of the skin surface being calculated according to the formula of DuBois (6). The volume of sweat was taken as the sum of the free sweat delivered from the envelope *plus* the amount adhering to the inner surface of the envelope. The latter was determined by weighing the envelope before and after it had been wetted with sweat.

Temperatures of the room, water bath and skin were recorded by a Micromax apparatus. Separate electrodes were used in the determination of room and bath temperatures and of the skin enclosed in the rubber envelope. For the latter determination two electrodes were employed, one being fixed on the lateral surface of the arm just above the elbow and one on the dorsal surface of the forearm just above the wrist. Readings from each electrode were recorded every eight minutes during the period of collection. The reported temperature in each instance is the average of the individual readings. In these experiments, the intensity of thermal stimulation (see below) was arbitrarily taken to be the room temperature when the bath temperature was kept the same

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[1] This work was supported by a grant from the Commonwealth Fund of New York to the Department of Pediatrics and was aided in part by a grant from the Rockefeller Foundation to the Department of Dermatology, Harvard Medical School.

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(44.5 ± 0.5° C) during repeated collections of sweat, or the bath temperature when the room temperature was kept the same (32 ± 0.3° C).

After collection, the sweat was filtered through Whatman No. 3 filter paper. Chloride concentrations were determined by a modification of the Volhard titration method (7); sodium, by the method of Butler and Tuthill (8) or by flame photometry with an internal standard; potassium, by the method of Fiske and Litarzek (9) or by flame photometry with an internal standard.

## RESULTS

### I. *Relations between sweat chloride concentration, intensity of thermal stimulation, skin temperature and rate of sweating.*

Data concerning these relations are contained in Table I, Experiments 1, 2, 3a, 4a. The subjects for these experiments were four normal adult males. Five to seven samples of sweat were collected from each subject. Collections were usually made at daily intervals.

The data demonstrate that there were great differences in the concentration of chloride in the several samples of sweat collected from each subject. For example, in the case of subject 3 the minimum concentration was 6 mEq per L. and the maximum, 54 mEq per L. Coefficients of correlation (Table II) indicate that variations in

TABLE I

*Data illustrating the influence of certain non-hormonal and hormonal factors on sweat electrolyte concentrations*

| Exp. no. | Subj. no. | Sex and age | Diagnosis | Date | NaCl intake | Treatment | Room temp. | Bath temp. | Skin temp. | Rate of sweating | Sweat electrolytes (*mEq per L.*) | | | Comments |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | Cl | Na | K | |
| | | | | | *gms./$m^2$/day* | | *°C* | *°C* | *°C* | *ml.** | | | | |
| 1 | 1 | ♂25 | Normal | 5/16 | 5 | None | 32.0 | 45.8 | 36.7 | 35 | 39 | 47 | | |
| | | | | 5/17 | 5 | None | 31.7 | 41.5 | 36.3 | 12 | 16 | 21 | 8.7 | |
| | | | | 5/18 | 5 | None | 32.2 | 43.6 | 36.8 | 32 | 25 | 32 | 6.6 | |
| | | | | 5/19 | 5 | None | 32.0 | 39.7 | 36.3 | 11 | 7 | 11 | 8.6 | |
| | | | | 5/20 | 5 | None | 32.0 | 40.0 | 36.3 | 9 | 7 | 11 | | |
| 2 | 2 | ♂27 | Normal | 6/6 | 5 | None | 28.2 | 44.4 | 35.6 | 20 | 20 | 31 | 8.2 | |
| | | | | 6/7 | 5 | None | 33.2 | 44.6 | 36.6 | 44 | 42 | 54 | 7.0 | |
| | | | | 6/8 | 5 | None | 26.6 | 44.4 | 34.9 | 10 | 8 | 16 | 11.1 | |
| | | | | 6/9 | 5 | None | 33.7 | 45.0 | 36.7 | 47 | 39 | 54 | 7.6 | |
| | | | | 6/10 | 5 | None | 27.5 | 44.1 | 36.0 | 18 | 12 | 19 | 8.3 | |
| | | | | 6/11 | 5 | None | 35.5 | 44.5 | 37.5 | 54 | 43 | 60 | 7.1 | |
| 3a | 3 | ♂27 | Normal | 7/7 | 5 | None | 29.7 | 44.7 | 36.7 | 18 | 16 | 22 | 8.5 | |
| | | | | 7/8 | 5 | None | 31.8 | 44.3 | 36.8 | 33 | 24 | 28 | 7.0 | |
| | | | | 7/9 | 5 | None | 25.9 | 44.6 | 36.5 | 20 | 17 | 23 | 7.5 | |
| | | | | 7/10 | 5 | None | 37.3 | 44.5 | 37.7 | 71 | 54 | 62 | 6.4 | |
| | | | | 7/11 | 5 | None | 26.8 | 40.6 | 36.0 | 7 | 6 | 10 | 8.5 | |
| | | | | 7/12 | 5 | None | 33.8 | 44.7 | 37.5 | 55 | 35 | 39 | 6.5 | |
| | | | | 7/13 | 5 | D.C.A. 5 mgm. i.m. b.i.d. | 28.1 | 44.6 | 37.0 | 37 | 18 | 22 | 6.3 | D.C.A. started after sweat collection |
| 3b | 3 | | | 7/14 | 5 | D.C.A. 5 mgm. i.m. b.i.d. | 29.8 | 44.4 | 37.6 | 44 | 12 | 16 | 7.2 | |
| | | | | 7/15 | 5 | D.C.A. 5 mgm. i.m. b.i.d. | 38.3 | 44.6 | 37.8 | 49 | 25 | 32 | 6.8 | |
| | | | | 7/16 | 5 | D.C.A. 5 mgm. i.m. b.i.d. | 25.1 | 44.5 | 35.9 | 10 | 4 | 8 | 8.7 | |
| | | | | 7/17 | 5 | D.C.A. 5 mgm. i.m. b.i.d. | 33.4 | 44.7 | 37.3 | 62 | 20 | 25 | 6.7 | |
| | | | | 7/18 | 5 | D.C.A. 5 mgm. i.m. | 29.5 | 44.5 | 36.3 | 42 | 9 | 9 | 5.9 | |
| 4a | 4 | ♂26 | Normal | 9/12 | 5 | None | 29.8 | 44.4 | 36.6 | 15 | 21 | 28 | 7.3 | |
| | | | | 9/13 | 5 | None | 34.3 | 44.5 | 37.0 | 26 | 29 | 36 | 5.8 | |
| | | | | 9/14 | 5 | None | 26.2 | 44.4 | 36.6 | 14 | 14 | 19 | 6.6 | |
| | | | | 9/15 | 5 | None | 33.5 | 44.4 | 37.4 | 38 | 36 | 45 | 5.5 | |
| | | | | 9/16 | 5 | None | 25.8 | 42.6 | 35.1 | 9 | 11 | 15 | 7.7 | |
| | | | | 9/17 | 5 | None | | | | | | | | |
| | | | | 9/18 | 5 | None | | | | | | | | |
| | | | | 9/19 | 5 | None | 38.2 | 45.0 | 37.9 | 52 | 48 | 57 | 6.6 | |
| | | | | 9/20 | 5 | Progesterone 50 mgm. i.m. | 32.7 | 44.3 | 36.1 | 23 | 20 | | | Progesterone started after sweat collection |
| 4b | 4 | | | 9/21 | 5 | Progesterone 50 mgm. i.m. b.i.d. | 29.6 | 44.6 | 35.3 | 21 | 15 | | | |
| | | | | 9/22 | 5 | None | | | | | | | | |
| | | | | 9/23 | 5 | Progesterone 50 mgm. i.m. b.i.d. | 25.9 | 42.8 | 35.6 | 13 | 11 | | | |
| | | | | 9/24 | 5 | Progesterone 50 mgm. i.m. b.i.d. | 34.0 | 44.5 | 37.1 | 37 | 36 | | | |
| | | | | 9/25 | 5 | Progesterone 100 mgm. i.m. | | | | | | | | |
| | | | | 9/26 | 5 | None | 30.1 | 44.5 | 37.0 | 32 | 25 | | | |

TABLE I—*Continued*

| Exp. no. | Subj. no. | Sex and age | Diagnosis | Date | NaCl intake | Treatment | Room temp. | Bath temp. | Skin temp. | Rate of sweating | Sweat electrolytes (*mEq per L.*) | | | Comments |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | Cl | Na | K | |
| | | | | | *gms./m²/day* | | *°C* | *°C* | *°C* | *ml.** | | | | |
| 5a | 5 | ♂17 | Normal | 8/21 | 5 | None | 35.1 | 44.5 | | 14 | 8 | 13 | 9.1 | |
| | | | | 8/22 | 5 | None | 35.5 | 44.5 | | 29 | 12 | 19 | 5.5 | |
| | | | | 8/23 | 5 | ACTH 25 mgm. b.i.d. | 35.3 | 44.1 | | 34 | 16 | 22 | 4.4 | ACTH started after sweat collection |
| 5b | 5 | | | 8/24 | 5 | ACTH 25 mgm. q.i.d. | 35.9 | 44.4 | | 55 | 18 | 27 | 5.2 | |
| | | | | 8/25 | 5 | ACTH 25 mgm. q.i.d. | 36.0 | 44.4 | | 53 | 12 | 11 | 5.1 | |
| | | | | 8/26 | 5 | ACTH b.i.d. | 36.0 | 44.3 | | 53 | 10 | 13 | 5.7 | |
| 5c | 5 | | | 8/27 | 5 | None | 36.0 | 44.3 | | 54 | 11 | 16 | 5.4 | |
| | | | | 8/28 | 5 | None | 36.0 | 44.4 | | 55 | 16 | 19 | 5.0 | |
| | | | | 8/29 | 5 | None | 36.1 | 44.4 | | 54 | 18 | 21 | 5.4 | |
| 6a | 6 | ♂24 | Normal | 10/13 | 5 | None | 29.0 | 44.2 | | 17 | 12 | 22 | 8 | |
| | | | | 10/14 | 5 | None | 32.7 | 44.3 | | 29 | 19 | 23 | 5.5 | |
| | | | | 10/15 | 5 | Methyl testosterone 25 mgm. q.i.d. p.o. | 36.2 | 44.4 | | 32 | 23 | 27 | 5.1 | Testosterone started after sweat collection |
| | | | | 10/18 | 5 | Methyl testosterone 25 mgm. q.i.d. p.o. | | | | 20 | 18 | 26 | 6.1 | Treatment with methyl testosterone since 10/15 |
| | | | | 10/19 | 5 | Methyl testosterone 25 mgm. q.i.d. p.o. | | | | 12 | 13 | 19 | .7.1 | |
| | | | | 10/20 | 5 | None | | | | 37 | 34 | | 10.1 | |
| 7 | 6 | ♂24 | Normal | 3/10 | 5 | None | 30.5 | 44.4 | | 7 | 17 | 23 | 11.6 | |
| | | | | 3/11 | 5 | None | 35.7 | 44.7 | | 17 | 28 | 30 | 7.1 | |
| | | | | 3/12 | 5 | None | 36.6 | 44.4 | | 18 | 19 | 23 | 5.6 | |
| 8a | 7 | ♀25 | Normal | 7/24 | 5 | None | 37.1 | 45.2 | | 33 | 47 | 58 | 5.5 | |
| | | | | 7/25 | 5 | None | 36.9 | 44.7 | | 33 | 46 | 56 | 4.6 | |
| | | | | 7/26 | 5 | None | 36.4 | 44.4 | | 28 | 38 | 47 | 5.5 | |
| 8b | 7 | | | 7/27 | 5 | Estradiol benz. 5 mgm. i.m. | 36.4 | 44.1 | | 20 | 26 | 33 | 5.7 | |
| | | | | 7/28 | 5 | Estradiol benz. 5 mgm. i.m. | 35.9 | 44.7 | | 27 | 29 | 36 | 5.1 | |
| | | | | 7/29 | 5 | Estradiol benz. 5 mgm. i.m. | 36.4 | 44.5 | | 34 | 34 | 42 | 5.2 | |
| 9 | 7 | ♀25 | Normal | 12/10 | 5 | None | 37.3 | 44.2 | | 17 | 56 | 64 | 7.3 | |
| | | | | 12/11 | 5 | None | 37.0 | 44.0 | | 18 | 58 | 65 | 7.5 | |
| | | | | 12/12 | 5 | None | 36.3 | 43.9 | | 18 | 49 | 60 | 7.4 | |
| 10a | 8 | ♂34 | Normal | 4/29 | 5 | None | 33.4 | 44.7 | | 48 | 22 | 29 | 5.4 | Subject frequently exposed to high temperatures prior to acting as experimental subject |
| | | | | 4/30 | 5 | None | | | | | | | | |
| | | | | 5/1 | 5 | None | 33.2 | 44.6 | | 41 | 20 | 26 | 5.2 | |
| | | | | 5/2 | 5 | None | 32.4 | 44.0 | | 43 | 15 | 22 | 5.4 | |
| | | | | 5/3 | 5 | None | 32.8 | 44.2 | | 36 | 13 | 18 | 5.0 | |
| | | | | 5/4 | 4 | Cortisone 100 mgm. i.m. | 32.7 | 44.3 | | 36 | 14 | 16 | 4.5 | |
| 10b | 8 | | | 5/5 | 5 | Cortisone 100 mgm. i.m. b.i.d. | 33.3 | 44.3 | | 44 | 16 | 21 | 4.5 | |
| | | | | 5/6 | 5 | Cortisone 100 mgm. i.m. b.i.d. | 33.2 | 43.4 | | 51 | 19 | 20 | 4.5 | |
| | | | | 5/7 | 5 | Cortisone 100 mgm. i.m. | 34.3 | 43.7 | | 41 | 19 | 23 | 5.2 | |
| 10c | 8 | | | 5/8 | 5 | None | 32.7 | 44.2 | | 35 | 14 | 16 | 4.8 | |
| | | | | 5/9 | 5 | None | 33.1 | 44.0 | | 42 | 13 | 15 | 4.7 | |
| | | | | 5/10 | 5 | None | 33.2 | 44.2 | | 58 | 24 | 29 | 4.8 | |
| | | | | 5/11 | 5 | None | 33.3 | 44.4 | | 43 | 19 | 22 | 4.8 | |
| | | | | 5/12 | 5 | None | | | | | | | | |
| | | | | 5/13 | 5 | None | 32.7 | 44.0 | | 35 | 14 | 17 | 5.3 | |
| | | | | 5/14 | 5 | None | | | | 41 | 13 | 18 | 4.3 | |
| | | | | 5/15 | 5 | None | | | | | | | | |
| | | | | 5/16 | 5 | None | 32.9 | 44.1 | | 44 | 16 | 22 | 4.8 | |
| 10d | 8 | | | 5/17 | 10 | None | | | | | | | | |
| | | | | 5/18 | 15 | None | 33.0 | 44.2 | | 39 | 16 | 21 | 5.1 | |
| | | | | 5/19 | 15 | None | 33.0 | 44.0 | | 35 | 20 | 26 | 5.0 | |
| | | | | 5/20 | 15 | None | 33.5 | 44.4 | | 42 | 32 | 39 | 4.3 | |
| | | | | 5/21 | 15 | None | | | | | | | | |
| | | | | 5/22 | 15 | None | 33.5 | 44.4 | | 42 | 30 | 36 | 4.2 | |
| | | | | 5/23 | 15 | None | 33.7 | 44.2 | | 48 | 37 | 43 | 4.0 | |

TABLE I—*Continued*

| Exp. *no.* | Subj. *no.* | Sex and age | Diagnosis | Date | NaCl intake | Treatment | Room *temp.* | Bath *temp.* | Skin *temp.* | Rate of *sweating* | Sweat electrolytes (*mEq per L.*) | | | Comments |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | Cl | Na | K | |
| | | | | | *gms./m²/day* | | *°C* | *°C* | *°C* | *ml.** | | | | |
| 11a | 8 | ♂33 | Normal | 12/13 | 5 | None | | | | 20 | 21 | 27 | 6.2 | Subject frequently exposed to high temperatures prior to acting as experimental subject |
| | | | | 12/14 | 5 | None | | | | 29 | 22 | 28 | 6.3 | |
| | | | | 12/15 | 5 | None | 30.1 | 41.8 | | 17 | 11 | 15 | 5.2 | |
| 11b | 8 | | | 12/16 | 5 | K supplement | | | | | | | | 27 gms. K citrate, .745 gms. $K_2HPO_4$, 3.78 gms. $KH_2PO_4$, added to daily diet |
| | | | | 12/17 | 5 | K supplement | 33.5 | 43.8 | | 22 | 13 | 17 | 9.1 | |
| | | | | 12/18 | 5 | K supplement | 30.4 | 45.3 | | 27 | 14 | 17 | 7.6 | |
| | | | | 12/19 | 5 | K supplement | 28.8 | 41.7 | | 25 | 14 | 15 | 7.3 | |
| | | | | 12/20 | 5 | K supplement | 28.7 | 44.7 | | 21 | 11 | 13 | 6.4 | |
| | | | | 12/21 | 5 | K supplement | 28.3 | 41.5 | | 19 | 10 | 12 | 6.4 | |
| 11c | 8 | | | 12/22 | 5 | None | | | | | | | | |
| | | | | 12/23 | 5 | None | | | | | | | | |
| | | | | 12/24 | 5 | None | 29.9 | 44 | | 12 | 10 | 13 | 7.3 | |
| | | | | 12/25 | 5 | None | 29.4 | 44.4 | | 26 | 17 | 21 | 7.0 | |
| | | | | 12/26 | 5 | None | | | | | | | | |
| | | | | 12/27 | 5 | None | 28.1 | 45.5 | | 24 | 19 | 16 | 3.6 | |
| | | | | 12/28 | 5 | None | 33.7 | 44.9 | | 27 | 21 | 18 | 3.4 | |
| | | | | 12/29 | 5 | None | 24.5 | 45.0 | | 11 | 8 | 14 | 7.9 | |
| 12 | 9 | ♂24 | Normal | 5/25 | 15 | None | 39.6 | 44.4 | | 60 | 79 | 96 | 6 | Legs immersed in water bath |
| | | | | 5/25 | | None | 41.1 | 44.3 | | 44 | 72 | 83 | 5.2 | |
| | | | | 5/25 | | None | 34.9 | 44.0 | | 40 | 67 | 78 | 5.0 | |
| | | | | 5/25 | | None | 31.2 | 41.2 | | 15 | 43 | 54 | 5.5 | |
| | | | | 5/25 | | None | 31.0 | 42.9 | | 24 | 50 | 61 | 5.4 | |
| 13 | 10 | ♂24 | Normal | 6/21 | 24 | None | 41.7 | 44.0 | | 40 | 87 | 98 | 4.8 | Legs immersed in water bath |
| | | | | 6/21 | | None | 37.5 | 44.8 | | 17 | 75 | 88 | 4.2 | |
| | | | | 6/21 | | None | 36.5 | 41.5 | | 9 | 52 | 63 | 5.6 | |
| | | | | 6/21 | | None | 36.8 | 42.1 | | 25 | 74 | 81 | 4.2 | |
| | | | | 6/21 | | None | 36.8 | 40.3 | | 15 | 66 | 76 | 4.0 | |
| 14a | 11 | ♂50 | Addison's disease | 8/3 | 2 | D.C.A. pellets | 25.0 | 44.1 | | 16 | 20 | 28 | 6.8 | |
| | | | | 8/4 | 2 | D.C.A. pellets | 28.1 | 44.4 | 34.8 | 14 | 19 | 26 | 7.4 | |
| | | | | 8/5 | 7 | D.C.A. pellets | 33.6 | 44.3 | 36.6 | 18 | 26 | 31 | 6.4 | |
| 14b | 11 | | | 8/9 | 7 | D.C.A. pellets | 27.2 | 44.5 | 36.4 | 8 | 17 | 18 | 5.6 | Daily intake NaCl 7 gms./m²/day since 8/5 |
| | | | | 8/10 | 7 | D.C.A. pellets | 32.7 | 44.4 | 37.1 | 18 | 27 | 36 | 7.1 | |
| | | | | 8/11 | 7 | D.C.A. pellets | | | | 22 | 34 | 44 | 7.2 | |
| 15 | 12 | ♂47 | Addison's disease | 5/11 | 4–6 | None | 34.2 | | | 3.1 | 37 | | | Arm not immersed in water bath |
| | | | | 5/11 | | None | 37.3 | 44.2 | | 32 | 88 | 102 | 6 | |
| | | | | 5/12 | | None | 30.3 | 44.1 | | 15 | 62 | | | |
| 16 | 13 | ♂48 | Essential hypertension | 2/24 | 5 | None | 37.2 | 44.3 | | 21 | 68 | 81 | 6.4 | Daily intake NaCl 5 gms./m²/day since 2/15 |
| | | | | 2/25 | 5 | None | 30.8 | 44.5 | | 17 | 48 | | 7.6 | |
| | | | | 2/26 | 5 | None | 32.8 | 44.4 | | 19 | 53 | 67 | 6.6 | |
| 17 | 14 | ♂53 | Essential hypertension | 3/27 | 5 | None | 31.2 | 44.4 | | 13 | 5 | 11 | 7.0 | Daily intake NaCl 5 gms./m²/day since 3/18/50 |
| | | | | 3/28 | 5 | None | 34.7 | 44.3 | | 19 | 7 | 13 | 6.1 | |
| | | | | 3/29 | 5 | None | 36.7 | 44.2 | | 26 | 10 | 16 | 6.3 | |

* *Ml. per hour per 0.1 m² of skin surface.*

TABLE II

*Coefficients of correlation**

| Factors correlated | Exp. no. (Table I) | | | |
|---|---|---|---|---|
| | 1 | 2 | 3a | 4a |
| Rate of sweating vs. sweat chloride concentration | +.933 | +.979 | +.963 | +.972 |
| Intensity of stimulation vs. sweat chloride concentration | +.996 | +.978 | +.923 | +.901 |
| Skin temperature vs. sweat chloride concentration | +*.829* | +.890 | +.919 | +.892 |
| Rate of sweating vs. intensity of stimulation | +.998 | +.998 | +.879 | +.887 |
| Rate of sweating vs. skin temperature | +.969 | +.947 | +.971 | +.861 |
| Intensity of stimulation vs. skin temperature | +*.860* | +.936 | +.886 | +*.678* |

* All coefficients are statistically significant ($p < 0.05$) except those italicized.

sweat chloride concentration were in each subject significantly correlated with *a*) rate of sweating, *b*) skin temperature and *c*) intensity of the thermal stimulus. In addition, they show that rate of sweating, skin temperature and the intensity of the thermal stimulus were significantly correlated with each other in almost all instances.

These findings point to a presumably nonhormonal factor capable of profoundly modifying the concentration of chloride in sweat obtained from normal subjects receiving a constant intake of sodium chloride. Consequently, it would appear that the intensity of action of the factor should be taken into consideration if sweat electrolyte composition is to be used as an index of an individual's hormonal status. Consideration of the data suggests that this may be done reasonably satisfactorily by measuring the rate of sweating. Accordingly, there follows a more detailed consideration of the relation between sweat chloride concentration and rate of sweating.

### II. *Detailed study of the relation between sweat chloride concentration and rate of sweating.*

In all experiments summarized in Table I, data concerning this relation are recorded. Representative data drawn from this table (Experiments 3a, 3b, 13, 14b, 15) are presented graphically in Figure 1.

The experiments summarized in the graph were performed on *a*) a normal adult male, maintained on the standard diet, both before (Curve A) and

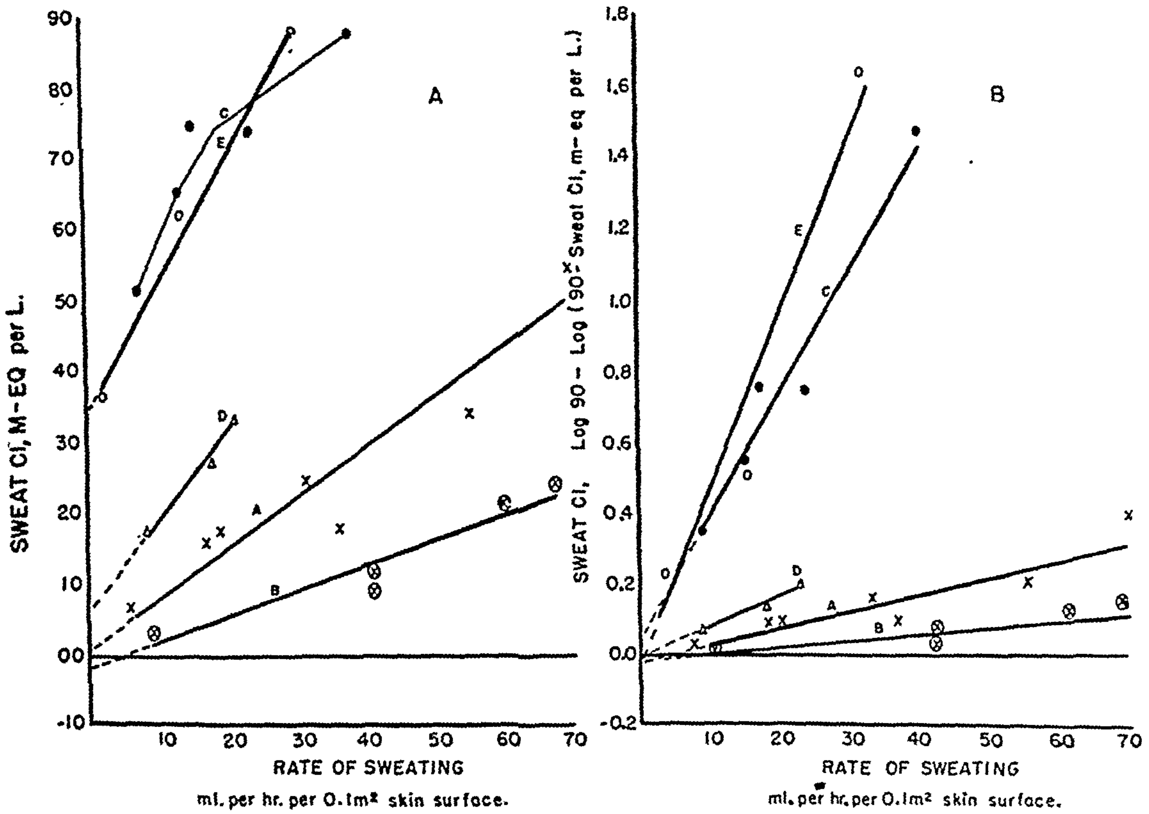

FIG. 1. RELATION BETWEEN SWEAT CHLORIDE CONCENTRATION AND RATE OF SWEATING

Data from Table I: Curve A—Experiment 3a; Curve B—Experiment 3b; Curve C—Experiment 13; Curve D—Experiment 14b; Curve E—Experiment 15. In both sections of the figure (A and B), absolute values for rate of sweating are indicated by the scale along the abscissa. In the left hand Section A, absolute chloride values are given according to the scale along the left hand ordinate. In the right hand Section B, the values indicated along the ordinate scale were found by subtracting from log 90 the log of (90 minus sweat chloride in mEq per L.). The heavy solid lines were calculated by the "method of least squares." The light solid line (Curve C, Section A) was drawn free-hand. Interrupted lines represent extrapolations of the heavy solid lines to the point where they would theoretically intersect the ordinate. The slopes of the solid lines of Section B define the value for the sweat chloride-rate index.

while he was being given desoxycorticosterone acetate (D.C.A.), 9 mgm. b.i.d., i.m. (Curve B); *b*) a normal male maintained on a diet which provided him with 24 gms. of sodium chloride per $m^2$ per day (Curve C); *c*) an Addisonian adult male whose diet contained 6.5 gms. of sodium chloride per $m^2$ per day and whose symptoms were well controlled by recently implanted pellets of D.C.A. (Curve D); and *d*) an Addisonian adult male whose diet contained approximately 5 gms. of sodium chloride per $m^2$ per day and whose symptoms were poorly controlled by 3 D.C.A. pellets implanted eight months previously (Curve E).

Figure 1 consists of two regression graphs, A and B. The same data are used in each and in both the abscissa is the rate of sweating and the ordinate is sweat chloride concentration. However, for reasons which will shortly become apparent, the methods for expressing chloride concentration differ.

In Figure 1-A chloride concentrations are expressed simply as mEq per L. of sweat. Note in this figure that in all instances there was an increase in the concentration of chloride as the rate of sweating increased. However, the properties of the curves describing the relation between the two variables appear to be very different. In four instances (Curves A, B, D and E) linear regression lines describe the relation satisfactorily but on extension they intercept the ordinate at widely different points (− 1.8 to + 33.2 mEq per L.). In the fifth instance (Curve C), the relation clearly is not linear.

Figure 1-A also shows that sweat chloride concentration measurements do not *per se* always permit one to distinguish between sweat from individuals subjected to different degrees of stimulation by the adrenal cortex or administered D.C.A. However, inspection of the figure reveals that such a distinction can be made if chloride concentration is viewed with relation to rate of sweating. Although evident on inspection, differences between the curves of Figure 1-A cannot be described and quantitated satisfactorily in simple arithmetic terms.

Because it seemed highly desirable to have a generally applicable, simple arithmetic method describing the relation between chloride concentration and rate of sweating, other methods of expressing the data were sought. Examination of available data suggested that the relation between sweat chloride concentration and rate of sweating might be described satisfactorily by a series of catalytic curves asymptotic at a chloride concentration of approximately 90 mEq per L. Accordingly, to test this possibility values for sweat chloride concentration were expressed as log 90 minus log (90 minus sweat chloride concentration in mEq per L.). The resultant values have been plotted (ordinate) against rate of sweating (abscissa) in Figure 1-B. Note that in this graph all curves are linear and all, on extension, intercept the ordinate very close to the origin. Differences between them consist only in differences in slope. This fact permits the relation between sweat chloride concentration and rate of sweating to be represented with fair accuracy by dividing the ordinate by the abscissa value. The magnitude of the resultant quotient is directly proportional to the slope of the curve. For convenience, this quotient, expressed as a simple number, will be called the *sweat chloride-rate index*. This index is used henceforth to indicate relations between chloride concentration and rate of sweating.

### III. *Relations between the concentration of chloride, sodium, and potassium in thermal sweat.*

In the preceding and following sections of this paper, attention is restricted to the response of chloride ion to the various stimuli which affect sweat electrolyte composition. The present section indicates the extent to which chloride concentration measurements are representative of sweat sodium and potassium.

Figure 2 demonstrates by means of regression graphs relations between the concentration of chloride and *a*) the concentration of sodium, *b*) the concentration of potassium and *c*) the ratio, (*a*)/(*b*), as determined in 57 different specimens of thermal sweat. Fifty-one of the 57 specimens were collected from normal subjects. Of these 35 came from subjects eating the standard diet. Twenty-three of the 35 specimens were collected from subjects who were not receiving hormonal treatment; the remaining 12 specimens were collected from subjects being treated with D.C.A. Seven specimens were collected from a subject whose daily diet contained a great excess of sodium over chloride (220 mEq Na, 12 mEq Cl)

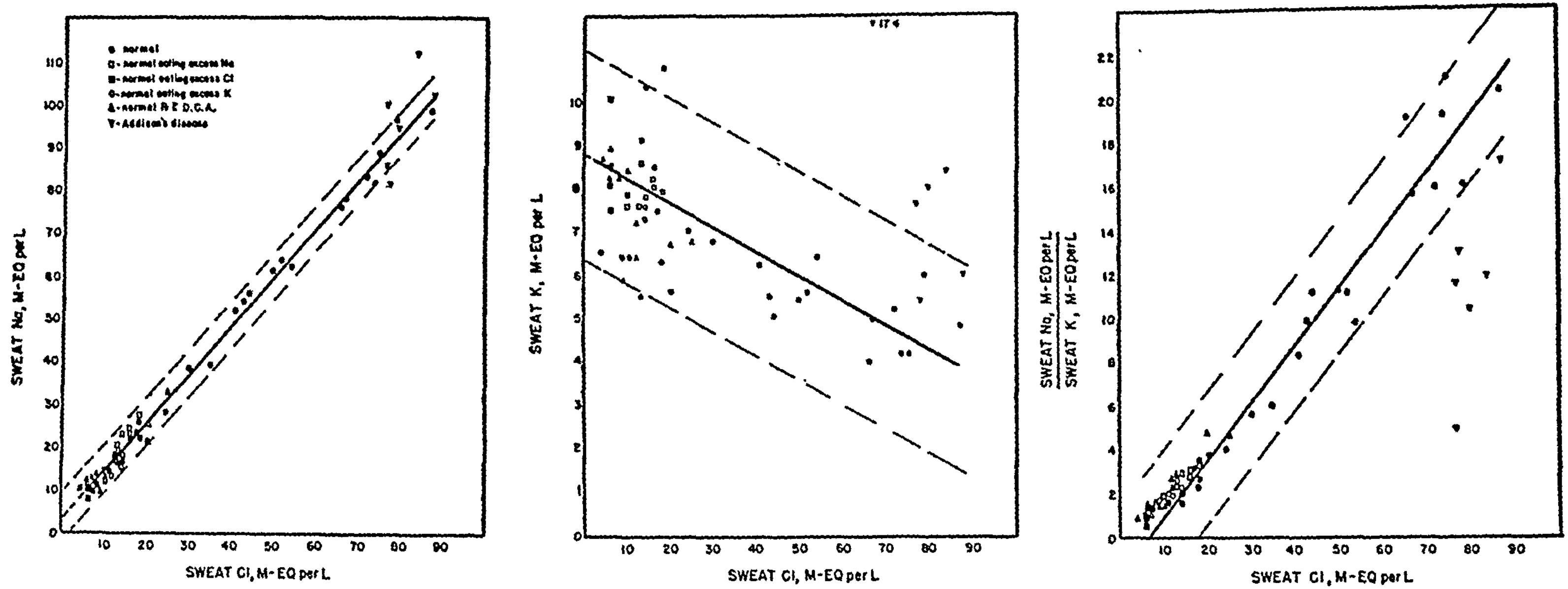

FIG. 2. RELATIONS BETWEEN THE CONCENTRATIONS OF CHLORIDE, SODIUM, AND POTASSIUM IN THERMAL SWEAT

The broken lines indicate limits of normal variability (y ± standard error of estimate).

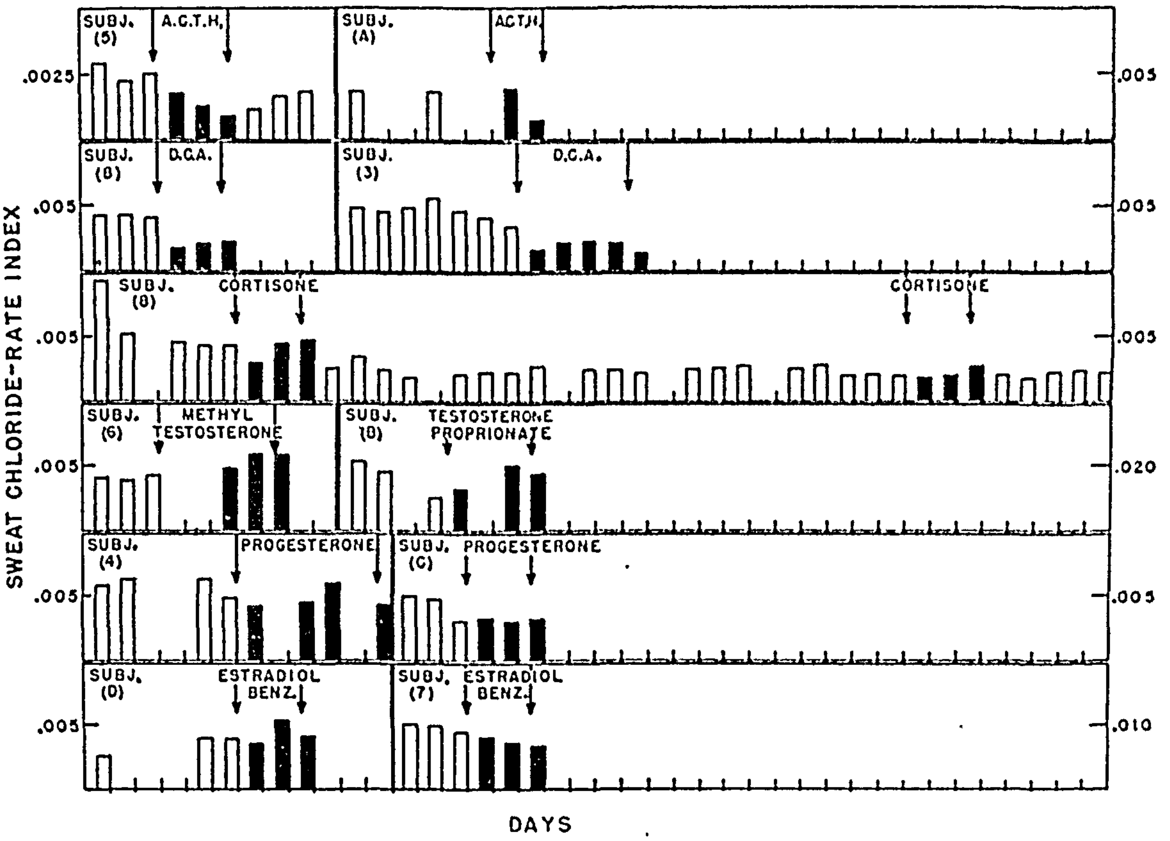

FIG. 3. THE EFFECT OF CERTAIN HORMONES ON THE SWEAT CHLORIDE-RATE INDEX

ACTH 25 mgm. q.i.d.; D.C.A. 5 mgm. b.i.d.; Cortisone 75 mgm. and 100 mgm. b.i.d.; Methyl testosterone 25 mgm. q.i.d.; Testosterone propionate 100 mgm. q.d.; Progesterone 50 mgm. b.i.d.; Estradiol benzoate 5 mgm. q.d. Subjects are designated by number if data are included in Table I.

and four were collected from the same subject when his daily diet contained a great excess of chloride over sodium (15 mEq Na, 220 mEq Cl). Five specimens were obtained from a subject whose basal (standard) daily diet was supplemented with large amounts of potassium salts (370 mEq K). Six specimens were collected from patients having poorly controlled Addison's disease.

In the sweats from the untreated normal subjects eating the standard diet, the relation between the concentration of chloride and each of the other three variables is linear. These relations, expressed mathematically were:

*a*) $[\mathrm{Na}] = 1.12\ [\mathrm{Cl}] + 3$ mEq per L. (standard error of estimate 2.6 mEq per L.)

*b*) $[\mathrm{K}] = 0.056\ [\mathrm{Cl}] + 8.8$ mEq per L. (standard error of estimate 1.2 mEq per L.)

*c*) $[\mathrm{Na}]/[\mathrm{K}] = 0.26\ [\mathrm{Cl}] - 1.7$ (standard error of estimate, 1.52).

The coefficients of correlation between chloride concentration and each of the other three variables are all significant. As the concentration of chloride rises the concentration of sodium and the ratio between the concentrations of sodium and potassium rise while the concentration of potassium falls.

The values for the 11 samples of sweat from the individual eating the diets grossly abnormal with respect to their electrolyte contents all fell within the range of normal variability.[3] In the 12 samples of sweat collected from individuals treated with D.C.A. all values fell within the "normal range" except for one value for potassium concentration which fell below the normal range. In the six samples of sweat obtained from patients having poorly controlled Addison's disease, in relation to the concentration of chloride, *a*) three sodium concentrations fell within, two fell above and one fell below the normal range, *b*) two potassium concentrations fell within and four above the normal range and *c*) all six ratios between the concentrations of sodium and potassium were below the normal range.

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[3] The normal range of variability was taken to be $y \pm 2$ standard error of estimate where y is [Na], [K] or [Na]/[K]. Such a range includes 96 per cent of determinations.

### IV. *Relations between sweat chloride index and hormonal status.*

A. *The effect of certain hormones on the sweat chloride-rate index.* In this series of experiments one subject, who received ACTH, had rheumatoid arthritis. All other subjects were normal. Cortisone acetate was given to one subject on two occasions. Methyl testosterone and testosterone propionate were each given to one subject. The other hormones were each given to two subjects. Methyl testosterone was taken by mouth. The other hormones were administered intramuscularly. The hormones and dosages employed were as follows: Adrenocorticotropic hormone (Armour Standard ACTH) 25 mgm. b.i.d. or q.i.d. (see Table I); desoxycorticosterone acetate (D.C.A.) (Percorten, Ciba) 5 mgm. b.i.d.; cortisone acetate (Cortone, Merck) 75 mgm. b.i.d. on one occasion and 100 mgm. b.i.d. on another; methyl testosterone (Oreton-M, Schering) 25 mgm. q.i.d.; testosterone propionate (Oreton, Schering) 100 mgm. daily; progesterone (Proluton, Schering )50 mgm. b.i.d.; estradiol benzoate (Progynon-B, Schering) 5 mgm. daily. In most instances the hormones were administered over a three to five day period.

Figure 3 represents the results of these experiments. The administration of either ACTH or D.C.A. was accompanied by striking reductions in the value of the index in all instances. The administration of methyl testosterone was accompanied by a significant rise in index values. While cortisone, progesterone and testosterone propionate had no statistically significant effect on the sweat chloride-rate index, inspection of the data suggests that their administration was associated with a slight rise in index values. It is possible that longer administration of cortisone would have resulted in a tendency to lowered index values. In one instance the administration of estradiol benzoate was accompanied by a significant reduction in the index; in the other instance this hormone had if anything an opposite effect.

B. *A comparison of the sweat chloride-rate index in normal and hypoadrenocortical subjects.* Figure 4 presents measurements of the sweat chloride-rate index obtained as a result of the first sweating in the cases of *a*) 10 normal subjects (seven male, three female) eating the standard diet during the summer; *b*) nine normal subjects (five male, four female) eating the standard diet during the winter; *c*) seven hypoadrenocortical subjects of which three were male and four female. Collections of sweat were made from the hypoadrenocortical patients during both seasons of the year. One of these patients ate the standard diet. The others were on hospital diets estimated to contain 4–6 gms. of sodium chloride per $m^2$ per day.

In the normal subjects eating the standard diet, the average value for the index was during the summer .0054 (range .0029 to .0097) and during the winter .0165 (range .0111 to .0249). In the hypoadrenocortical subjects the average was .1095 (range .0348 to .1809). In none of the three groups did the sex of the subject appear to affect the index value.

### V. *The sweat chloride-rate index in studies of adrenal function in man.*

A. *Effect of varying the dietary intake of sodium chloride and potassium salts.* Figure 5 portrays data obtained in experiments involving one normal male (Table I, Experiments No. 10c-d), one diabetic female and one patient having Addison's disease (Table I, Experiments No. 14a-b). Their dietary intakes of sodium chloride per day varied

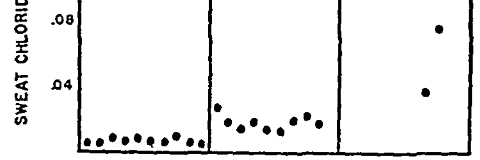

FIG. 4. SWEAT CHLORIDE-RATE INDICES FOR THE FIRST SWEATING OF NORMAL AND HYPOADRENOCORTICAL SUBJECTS

In the hypoadrenocortical group, black circles represent patients with primary adrenal cortical insufficiency and the single white circle represents a patient with hypoadrenocorticism secondary to hypopituitarism.

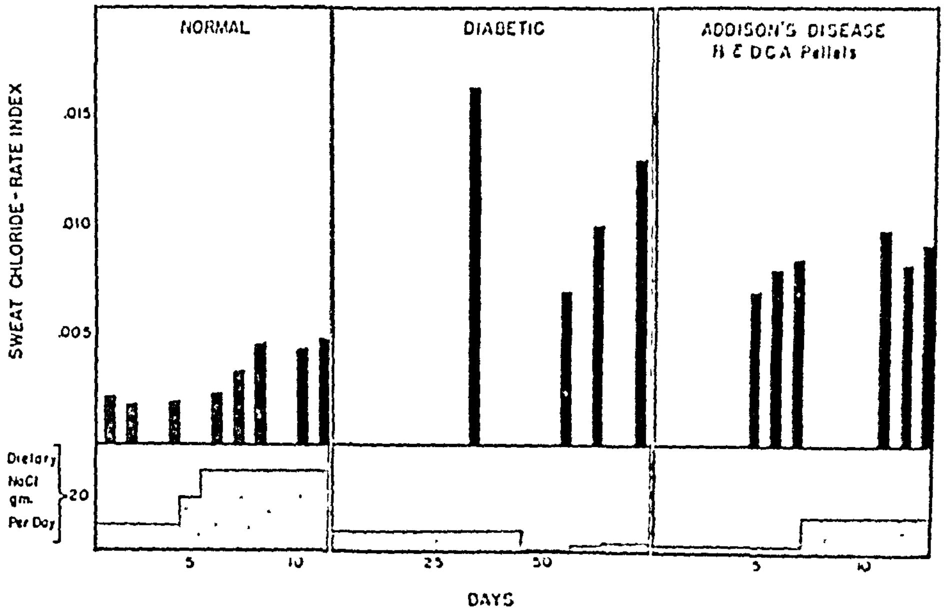

Fig. 5. The Effect on the Sweat Chloride-Rate Index of Varying Dietary Sodium Chloride

Differences noted in the normal and diabetic subjects following changes in dietary NaCl intake are statistically significant; the difference between the two groups of values obtained in the Addisonian patient is not statistically significant.

between 10 and 30 gms., 0.6 and 8.6 gms. and 3.5 and 11.5 gms., respectively. Note that the sweat chloride-rate index closely parallels the sodium chloride intake in the normal and in the diabetic subjects. In the Addisonian patient, no statistically significant change in the index value occurred.

In Experiments 12 and 13, Table I, two normal male subjects were given diets containing very large amounts of sodium chloride (15 and 24 gms. per $m^2$ per day, respectively) for 10 days. At the time of the first sweating the respective sweat chloride-rate indices were .0152 and .0369, the latter being within the range observed for patients with untreated Addison's disease. The range of indices in 10 comparable normal subjects on the standard diet is .0029 to .0097 (Figure 4).

Figure 6 describes the effect on the sweat chloride-rate index of a normal male of adding 27 gms. of potassium citrate, 8.02 gms. of dipotassium phosphate and 3.78 gms. of monopotassium phosphate (370 mEq of K) to a standard diet. Note that the index value was significantly lower during the high potassium period than during two control periods.

B. *Effects of exposure to heat.* Figure 7 describes the effect on the sweat chloride-rate index of normal subjects of *a*) season (see also Figure 4) and *b*) repeated sweating. It is evident that the indices are considerably lower during the "summer" (May 1 to October 31) than during the "winter" (November 1 to April 30). In the winter, but not in the summer, there is a well defined tendency for index values to fall when the subject

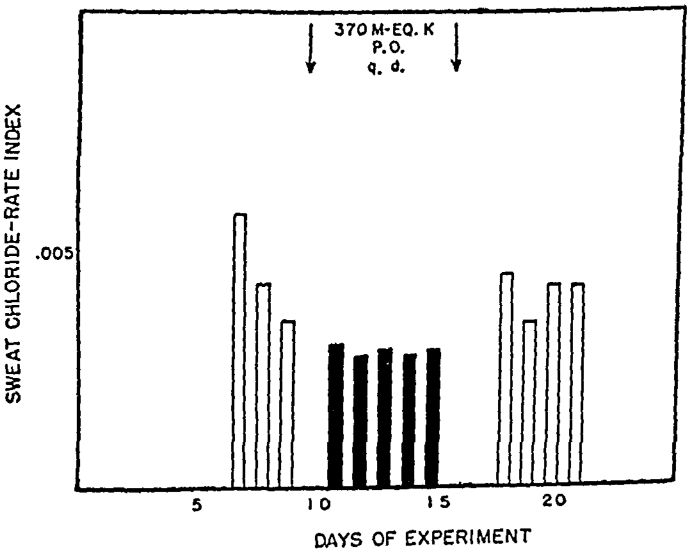

Fig. 6. The Effect on the Sweat Chloride-Rate Index of Increasing the Dietary Intake of Potassium Salts

The values during the high potassium period are significantly different from the control values.

is sweated repeatedly on consecutive days. The observations of Figure 7 plus other incomplete data suggest that between three and approximately eight consecutive days of hourly exposure to intense heat are required to induce a drop in index values from average winter to average summer levels.

C. *Hypertension.* Figure 8 shows the sweat chloride-rate indices of four patients suffering idiopathic hypertension. These measurements were carried out during the winter. The patients had been maintained on diets low in sodium (estimated to contain about 1 gm. of sodium chloride per $m^2$ per day) for from five to 20 months prior to the beginning of the experiments. For the first 10 days of the experiment, during which no sweat was collected, and for the subsequent two to three days during which collections were made at daily intervals, they were maintained on the standard diet.

It is seen that sweat chloride-rate indices for two of the patients fell within the normal range (Figure 7); in the other two patients the values definitely were subnormal.

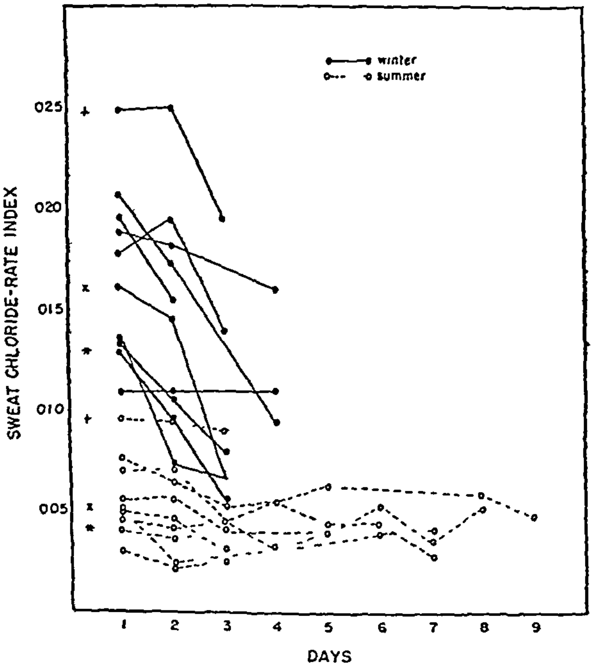

FIG. 7. THE EFFECT OF SEASON AND REPEATED SWEATINGS ON THE SWEAT CHLORIDE-RATE INDEX

At the left of some of the curves are symbols. Each type of symbol represents a single individual. Thus repeat studies on the same individual can be recognized by symbol duplication

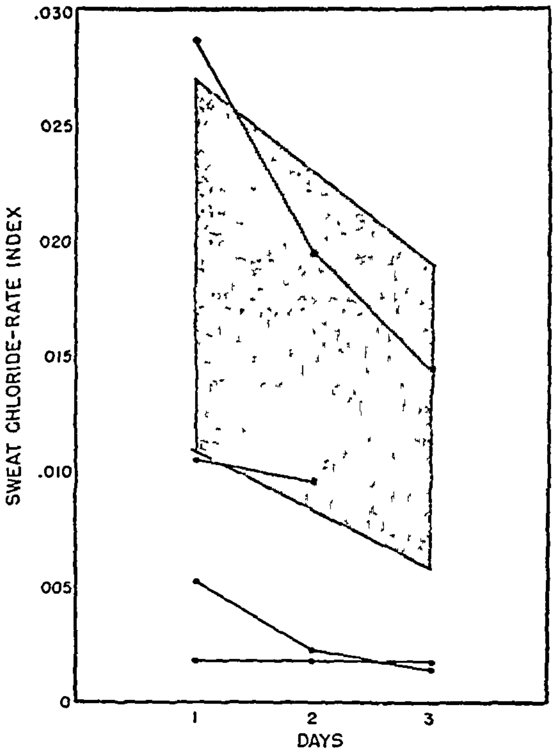

FIG. 8. VALUES FOR THE SWEAT CHLORIDE-RATE INDEX OF FOUR HYPERTENSIVE SUBJECTS

These values were obtained during the winter. The shaded area gives the range of values obtained on normal subjects during the same season as indicated in Figure 7.

### DISCUSSION

The initial studies described in this report show clearly that sweat electrolyte composition is influenced almost as much by non-endocrine factors related to intensity of thermal stimulus, skin temperature and rate of sweating as by active adrenal cortical hormones. Under conditions of constant hormonal status, sweat electrolyte concentrations bear a mathematically predictable relation to these non-endocrine variables. A change in intensity of action of hormones which influence sweat formation results in an alteration in sweat electrolyte composition relative to thermal stimulus, skin temperature and rate of sweating.

The sweat chloride-rate index was evolved in an effort to express such changes in sweat electrolytes relative to these non-hormonal factors in a practically useful manner. Values for the concentration of chloride bear a closely predictable direct relation to those of sodium and an approximate, inverse relation to those of potassium in sweat. Hence chloride may be considered satisfactorily representative of the other two sweat electrolytes. Rate of sweating does not always bear a close relation to intensity of thermal stimulus or skin temperature. On the other hand, it usually is roughly proportional to these variables. Rate of sweating is closely correlated with sweat chloride concentration when the dietary and hormonal status are maintained constant. Because of this and the fact that rate of sweating is readily measureable, it was selected to represent the nonendocrine factors in the index equation.

The observations on hypoadrenocortical patients coupled with the hormone administration studies provide clues concerning the probable nature of endocrine factors responsible for changes in sweat chloride-rate index values. Index values were consistently higher than normal in hypoadrenocortical patients. Desoxycorticosterone acetate was the only one of the steroid hormones to induce a marked decrease in the index value. This occurred within one day. Interestingly, 17-hydroxy-11-dehydrocorticosterone (cortisone) induced if anything a slight rise in index values over a period of three days.

These data suggest that sweat chloride-rate index values bear an inverse relation to the concentration of adrenal cortical hormones which, like desoxycorticosterone, act chiefly upon body electrolyte and water metabolism (10). Adrenocorticotropic hormone induced as great and as rapid a fall in the index value of normal subjects as did desoxycorticosterone acetate. It therefore appears that the human adrenal cortex secretes a hormone with desoxycorticosterone-like properties in response to adrenocorticotropin stimulation.

While the estrogens and androgens given were not wholly without effect upon sweat chloride-rate index values, they did not appear to exert a striking and consistent regulatory influence. This suggests that their influence upon sweat formation may have been accomplished indirectly by stimulating or inhibiting pituitary adrenocorticotropic hormone production rather than by direct action upon the sweat glands. Inhibition of adrenocorticotropic hormone secretion and hence of adrenal cortical activity might account for the slight rise in index values observed following cortisone, progesterone and testosterone. This thesis is compatible with the fact that cortisone and methyl testosterone act to suppress adrenal cortical urinary 17-ketosteroid precursor production, presumably by inhibiting adrenocorticotropin secretion (11, 12). Methyl testosterone also can inhibit 11-17-oxycorticosteroid excretion (13). The single observation that estradiol may induce a lowering of index values likewise is in keeping with the fact that estrogens can cause adrenal cortical hyperplasia by stimulating secretion of adrenocorticotropic hormone (14).

The foregoing considerations lead to the conclusion that sweat chloride-rate index values are determined chiefly by adrenal cortical hormones of the 11-desoxycorticosterone type. A rise in index values therefore suggests a decrease, and a fall in index values an increase in adrenal cortical activity.

The present studies of adrenal cortical status based on measurements of this index extend and confirm observations of other workers which suggest that the adrenal cortices undergo physiologic alterations in activity in response to certain situations (14–16). Thus the consistent tendency for the sweat chloride-rate index to fall when normal persons are subjected to low sodium chloride diets, high potassium diets or repeated exposure to heat indicate that these factors induce a physiologic increase in adrenal cortical activity. By contrast, high sodium chloride diets prompt the index values of normal persons to rise to levels comparable to those observed in patients with primary hypoadrenocorticism. The fact that no significant changes in index values took place in a hypoadrenocortical patient when the salt intake was varied indicates that the changes observed in normal subjects were dependent upon the presence of functionally intact adrenal cortices.

The comparative observations on the normal subjects taking a high sodium diet and on the hypoadrenocortical patients indicate that under certain circumstances absolute rates of adrenal cortical secretory activity attained physiologically may closely approximate those observed in patients with adrenal cortical pathology. Similar information is provided by studies of urinary 11-17-oxycorticosteroid excretion in health and disease (17). In other words, pathologic is distinguished from physiologic adrenal cortical function by the relation of secretory activity to physiologic, homeostatic needs rather than by absolute differences in secretory rate.

Preliminary observations on patients with idiopathic hypertension indicate that the sweat chloride-rate index may provide interesting diagnostic information in certain presently obscure diseases. Two of the four patients studied had values suggestive of pathologic hyperproduction of the electrolyte-water regulating type of adrenal cortical hormone.

## SUMMARY AND CONCLUSIONS

1. Intensity of thermal stimulus, skin temperature and rate of sweating bear an important relation to sweat electrolyte composition. Certain hormones act to modify sweat composition relative to these factors. Such hormonal action can be appraised by use of an index which expresses sweat chloride concentration values relative to rate of sweating.
2. Studies suggest that the chief determinant of sweat chloride-rate index values is an adrenal cortical hormone which, like desoxycorticosterone, acts chiefly upon electrolyte and water metabolism.
3. Measurements of the sweat chloride-rate index suggest strongly that the normal adrenal cortices can undergo wide variations in activity in response to physiologic, homeostatic needs. Under physiologic conditions, adrenal cortical production of desoxycorticosterone-like hormones may subside to levels comparable to those existing in patients with primary pathologic hypoadrenocorticism.
4. The possibility that the sweat chloride-rate index measurement may have diagnostic value in miscellaneous conditions is exemplified by preliminary observations on four patients with idiopathic hypertension.

## ACKNOWLEDGMENTS

The supplies of cortisone used in these studies were generously provided by Merck and Co. We are indebted to the Schering Corporation for the other steroids and to the Armour Co. for the ACTH used in these investigations. We also wish to acknowledge our gratitude to Drs. C. Frazier and I. H. Blank of the Dermatology Service of the Massachusetts General Hospital for the use of their air conditioned room and micromax apparatus.

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# THE EFFECT OF LIPIODOL ON ALVEOLAR GAS EXCHANGE[1, 2]

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(Submitted for publication October 16, 1950; accepted, January 2, 1951)

Within the past 15 years the advances in intrathoracic surgery have made visualization of the bronchial tree with contrast media, particularly iodized poppyseed oil (lipiodol), a common practice. Despite the established safety of the procedure in the vast majority of instances, repeated questions have been raised as to the innocuousness of the oil in the pulmonary parenchyma. The feeling has been expressed by some authors that it is unwise to perform any intrathoracic procedure for several weeks to several months following the administration of lipiodol. Thus Churchill (1) has advised delaying operation for a minimum of six weeks after bronchography because of fear of postoperative atelectasis and pneumonitis due to the oil. Belsey (2) was of the same opinion and a more recent paper by Miscall (3) indicates a persistence of this feeling.

Pinkerton (4) has concluded from animal experiments that lipiodol in the absence of infection produces almost no reaction in the lungs. In clinical use, however, some evidence of lipoid granuloma has been observed (5).

It is also conceivable that lipiodol in the alveoli might impair transalveolar gas exchange and thus interfere with recovery from an intrathoracic operation. Whereas immediately after introduction the material is limited chiefly to the bronchial tree, soon thereafter its presence can be demonstrated diffusely filling the lung parenchyma where it may persist for several weeks or longer. In an attempt to evaluate the possibility of deficiencies of transalveolar diffusion following the administration of lipiodol the present study was undertaken.

## MATERIAL AND METHODS

Ten patients who were to have lipiodol bronchograms were studied before, within several hours after the instillation of the oil and on the following day. Bilateral bronchograms were performed by the aspiration technique using 10 cc. of oil on each side. Since there is considerable puddling in the pyriform sinuses less than this actually enters the lungs. Nevertheless, with this method satisfactory mapping of the entire bronchial tree is regularly obtained. It should be stressed that a significant amount of residual lipiodol was seen in the alveoli of all patients on fluoroscopy at the time the last tests were performed.

The techniques employed to study the patients were: *1)* determination of the oxygen pressure ($P_{O_2}$) gradient between the alveolar air ($P_{A_{O_2}}$) and the arterial blood ($P_{a_{O_2}}$)[4]; *2)* measurement of the arterial oxygen saturation; *3)* determination of the time required to attain full saturation when the subject was switched from room air to 100% oxygen and *4)* determination of vital capacity and its subdivisions.

The patient was brought to the laboratory without medication, but not necessarily in fasting condition. After resting 10–15 minutes on a stretcher in the supine position a sample of arterial blood was withdrawn from the femoral artery in the course of one to two minutes, during which period the expired air was collected in a Douglas bag, which previously had been washed out twice with the same gas. The patient was provided with a mouthpiece and nose clip and a system of valves with small dead space was used for the air collection.

Precautions were taken to disturb the patient as little as possible. Thorough novocaine infiltration preceded the arterial puncture, and several minutes were allowed to elapse between the arterial puncture and the collection of blood and expired air. Twenty cc. of blood were collected in a lightly oiled syringe containing 0.5 cc. of a mixture of eight parts of commercial heparin solution and two parts of a saturated solution of sodium fluoride.

The direct determination of oxygen and carbon dioxide tensions of this blood sample was then immediately performed by a technique which has been described in detail elsewhere (7). In principle, it consists of the equilibra-

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[1] A preliminary report was published in the American College of Surgeons Forum on Fundamental Surgical Problems, 1950.

[2] This study was supported by a grant from the United States Public Health Service.

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[4] The symbols used throughout this publication have been made to conform with those recommended at the April, 1950, meeting of the American Physiological Society (6). Substitutions for formerly used symbols are as follows: 'P' is gas pressure in general; 'F' is fractional concentration in dry gas phase; 'I' is inspired gas; 'A' is alveolar gas; 'a' is arterial.

tion of 17 cc. of blood with an 80 cu. mm. gas bubble (initial composition of the bubble 12.15% $O_2$, 5.25% $CO_2$) for 10 minutes at 37° C. At the end of this time pressure equilibration of the respiratory gases in blood and gas phases is obtained with less than 1 mm. Hg change in gas pressure in the liquid phase. The bubble is then transferred to a Scholander micro gas analyzer, where its composition is determined. On the assumption that the total gas pressure of the bubble is equal to atmospheric, the partial pressures in the bubble, and therefore, in the blood, are thus found. This assumption is justified in the case of normal arterial blood. Though in some of the patients studied the total arterial gas pressure was probably somewhat less than atmospheric, this would not affect the partial pressures of $CO_2$ and $O_2$ to a significant degree. The error of this method, as found by performing the above determination on blood of known gaseous composition, amounts to + 0.1 ± 3.3 mm. Hg for $O_2$ and − 5.3 ± 2.4 mm. Hg for $CO_2$. A correction of + 3.0 mm. Hg has been made for the $CO_2$ pressure due to the diluting effect of the anticoagulant

The "ideal" alveolar oxygen pressure was determined from analysis of the expired air, collected simultaneously with the blood, and from the directly determined arterial $CO_2$ pressure. As defined by Riley and associates (8, 9) and Rahn (10) the "ideal" or "effective" alveolar pressures are those which if present continuously and uniformly in all functioning alveoli, and if complete equilibrium were reached between the blood and gas phases in the lung, would permit $CO_2$ and $O_2$ exchange in amounts equal to that measured from analysis of inspired and expired air. As alveolar ventilation-perfusion relationships vary in different parts of the normal, and particularly of the pathologic lung, and as equilibrium of oxygen between blood and alveolar air in the abnormal lung often is not accomplished, "ideal" alveolar air must remain a theoretical quantity. Its practical usefulness lies in the fact that reproducible values can be assigned to alveolar oxygen and carbon dioxide pressures, whereas this would be impossible to accomplish through direct sampling in the many instances of non-homogeneous alveolar air. The calculation of the "ideal" alveolar $P_{O_2}$ is based on the assumptions that the alveolar respiratory quotient equals that of expired air, and that the alveolar $CO_2$ tension equals that of arterial blood. Whereas the former assumption seems to be correct (10), the latter may not be strictly true in cases of severe pulmonary disease. However, the patients studied in this paper probably did not have a significant arterial-alveolar $P_{CO_2}$ gradient.

The equation used to calculate "effective" alveolar $P_{O_2}$ was the following (9, 11):

$$P_{A_{O_2}} = P_{I_{O_2}} + \frac{P_{a_{O_2}} \times F_{I_{O_2}}(1 - RQ)}{100 \times RQ} - \frac{P_{a_{CO_2}}}{RQ}.$$

As various techniques are in use for the direct determination of blood gas tensions and for the determination of alveolar air composition, the oxygen gradient in 10 healthy male subjects from 25–35 years of age was determined, using the technique described above. The results are summarized in Table I. The average gradient was 3.0 mm. Hg with a standard deviation of 2.2. Lilienthal and co-workers (12) using the same method for effective alveolar air and the Riley technique for direct measurement of the arterial gas tensions found an average gradient of 8.9 mm. Hg ± 2.9 in six normal subjects. Cournand and colleagues (13) with identical methods in 10 normal individuals found an average gradient of 10.0. Comroe and Dripps (14), employing a method for determining blood gas tensions comparable to ours and end-expiratory Haldane-Priestley alveolar air samples, found an average gradient of 0.7 ± 5.3. Barker, Lambertsen and associates (15), using effective alveolar air and an improved Riley method for arterial $P_{O_2}$ found an average difference between the two of 3.7 mm. Hg which was statistically not significant.

TABLE I

*Normal alveolar-arterial $P_{O_2}$ gradient**

| Case | Barometric pressure | Arterial $P_{CO_2}$ ($P_{aCO_2}$) | A Alveolar $P_{O_2}$ ($P_{A_{O_2}}$) | B Arterial $P_{O_2}$ ($P_{a_{O_2}}$) | A − B Gradient |
|---|---|---|---|---|---|
| | *mm. Hg* | *mm. Hg* | *mm. Hg* | *mm. Hg* | *mm. Hg* |
| 1 | 740 | 41.2 | 98.8 | 93.8 | 5.0 |
| 2 | 741 | 37.2 | 96.0 | 90.2 | 5.8 |
| 3 | 750 | 35.4 | 102.2 | 99.8 | 2.4 |
| 4 | 750 | 37.1 | 102.4 | 99.8 | 2.6 |
| 5 | 750 | 38.2 | 99.2 | 95.0 | 4.2 |
| 6 | 742 | 40.0 | 99.5 | 100.0 | −0.5 |
| 7 | 747 | 36.6 | 108.4 | 104.4 | 4.0 |
| 8 | 745 | 36.9 | 102.2 | 98.0 | 4.2 |
| 9 | 745 | 38.2 | 100.5 | 101.5 | −1.0 |
| 10 | 747 | 41.4 | 101.5 | 98.0 | 3.5 |

Av. 3.0 ± 2.2

* Normal males 25–35 years old.

*Arterial oxygen saturation*

Immediately following the collection of the blood sample for the direct determination of gas tensions a second syringe, the dead space of which was filled with heparin, was attached to the needle and 20 cc. withdrawn for the determination of oxygen saturation in duplicate. The manometric method of Van Slyke was used for oxygen content and the Sendroy technique for capacity (except in the first three patients in whom the usual tonometer method for capacity was employed). Duplicate determinations checked within 0.15 vol.%.

*Vital capacity and subdivisions*

The measurements of vital capacity, inspiratory capacity and expiratory reserve volume (6) were obtained with the patient supine. Three determinations of each volume were made and the largest value recorded All volumes shown in Table III are corrected to 37° C., prevailing barometric pressure and saturated with water vapor (BTPS).

*Saturation time from room air to 100% oxygen*

The time in seconds required for the arterial oxygen saturation to reach a maximum value when the inspired gas was changed from room air to 100% oxygen was de-

TABLE II

*Alveolar-arterial $P_{O_2}$ gradient before and after lipiodol*

| Case Age Sex | Diagnosis | Time | $O_2$ Saturation | $CO_2$ Content | Arterial $P_{CO_2}$ ($P_{aCO_2}$) | A Alveolar $P_{O_2}$ ($P_{AO_2}$) | B Arterial $P_{O_2}$ ($P_{aO_2}$) | A−B $P_{O_2}$ Gradient |
|---|---|---|---|---|---|---|---|---|
| | | | % | *mMol/L* | *mm. Hg* | *mm. Hg* | *mm. Hg* | *mm. Hg* |
| Ha<br>45<br>M | No pulmonary disease | Before | 99.27* | 19.29 | 43.0 | 88.8 | 90.3 | −1.5 |
| | | 5 hours | 94.67* | 17.81 | 41.6 | 99.7 | 95.2 | 4.5 |
| | | 1 day | 95.52* | 20.74 | 43.7 | 93.9 | 94.2 | −0.2 |
| Co<br>52<br>M | Bronchitis and slight emphysema | Before | 97.68* | 22.16 | 40.5 | 95.6 | 93.8 | 1.8 |
| | | 6 hours | 94.05 | 21.74 | 38.5 | 96.6 | 92.9 | 3.7 |
| | | 1 day | 96.62 | 21.43 | 38.8 | 90.6 | 90.4 | 0.2 |
| Bo<br>24<br>M | Bronchiectasis LLL and lingula | Before | 99.78 | 20.74 | 36.8 | 103.3 | 102.6 | 0.7 |
| | | 4 hours | 98.00 | 21.29 | 36.1 | 100.4 | 97.2 | 3.2 |
| Beh<br>17<br>F | No pulmonary disease | Before | 97.06 | 18.95 | 39.9 | 92.4 | 96.0 | −3.6 |
| | | 5 hours | 94.12 | 19.56 | 46.4 | 81.2 | 80.5 | 0.7 |
| | | 1 day | 92.68 | 21.88 | 47.7 | 91.7 | 89.0 | 2.7 |
| Bu<br>48<br>M | Carcinoma obstructing R. bronchus | Before | 85.06* | 27.22 | 46.9 | 92.2 | (48)† | 44.2 |
| | | 2 hours | 86.38* | 25.58 | 41.4 | 95.2 | 53.2 | 42.1 |
| | | 1 day | 84.95* | 27.75 | 45.6 | 94.9 | 56.1 | 38.7 |
| Bet<br>41<br>M | Severe emphysema | Before | 90.99* | 22.48 | 36.9 | 98.7 | 62.3 | 36.4 |
| | | 3 hours | 85.32* | 25.73 | 38.5 | 98.8 | 57.4 | 41.4 |
| | | 1 day | 91.13* | 23.62 | 36.5 | 95.8 | 60.3 | 35.5 |
| Ho<br>56<br>M | Bronchiectasis L. lung, fibrosis and severe emphysema | Before | 93.57 | 24.20 | 40.6 | 91.9 | 81.2 | 10.7 |
| | | Before | 91.03 | 24.40 | 42.0 | 92.3 | 81.6 | 10.7 |
| | | 7 hours | 86.87 | 24.84 | 42.1 | 95.1 | 52.8 | 42.3 |
| | | 1 day | 88.65 | 24.15 | 36.4 | 99.9 | 60.8 | 39.1 |
| | | 2 days | 92.95 | 23.30 | 42.5 | 89.8 | 89.9 | −0.1 |
| McC<br>20<br>M | Bronchiectasis LLL and lingula; minimal RLL | Before | 94.20 | 21.71 | 41.5 | 94.9 | 83.1 | 11.8 |
| | | 5 hours | 94.59 | 21.90 | 37.3 | 100.6 | 82.7 | 17.9 |
| | | 1 day | | | 42.8 | 93.1 | 83.9 | 9.2 |
| Da<br>15<br>F | Infected cysts LLL | Before | 93.41 | 24.26 | 43.3 | 92.3 | 85.8 | 6.5 |
| | | Before | — | — | 44.3 | 98.5 | 85.7 | 12.8 |
| | | 5½ hours | 92.40 | 22.26 | 44.3 | 89.2 | 82.2 | 7.0 |
| | | 1 day | 95.28 | 22.40 | 42.9 | 90.4 | 89.8 | 0.6 |
| Mo<br>69<br>M | Bronchiectasis R. lung | Control | 87.56 | 25.95 | 43.9 | 77.8 | 49.0 | 28.8 |
| | | Control | 92.39 | 23.52 | 42.1 | 104.0 | 58.9 | 45.1 |
| | | 4 hours | 81.60 | 25.00 | 42.9 | 91.1 | 52.5 | 38.6 |
| | | 1 day | — | — | 46.7 | 89.7 | 56.0 | 33.7 |

* Tonometer equilibration for capacity.

† Calculated from saturation as direct determination not satisfactory.

termined with a modified oximeter designed in this laboratory by Elam, Sleator and co-workers (16–18). The earpiece of this apparatus employs a tungsten filament light source and two selenium photocells from which the electrical responses are channeled separately. The first cell which receives transmitted light filtered through a Wratten 29 filter measures changes in the red spectral region and is sensitive to changes both in total hemoglobin content and in oxygen saturation. The infra-red light which reaches the other cell has passed through three thicknesses of Wratten 87 and two thicknesses of Wratten 88. The output of this cell reflects chiefly changes in the total amount of blood in the path and is independent of oxygen saturation above 60%. From the amplified and continuously recorded outputs of the two cells the oxygen saturation of the blood in the optical path can be determined by means of a calibration curve. This instrument is similar in principle to that described by Wood and Geraci (19). The lag of the recorder for full scale deflection is 0.45 seconds. The ear lobe to which the instrument was attached was "arterialized" by histamine iontophoresis using 5 milliamperes of current for one minute on both the anterior and posterior surfaces.

With the amplifications of the two channels set at an arbitrary level that would give an adequate differential between room air and 100% oxygen values the supine subject was allowed to breathe room air through a three-way stopcock and a system of one-way valves using a

TABLE III

*Effect of lipiodol on lung volumes*

| Case | Time | Inspiratory capacity | Expiratory reserve volume | Vital capacity | Vital capacity change |
|---|---|---|---|---|---|
| | | cc. | cc. | cc. | % |
| Ha | Before | 3,385 | 1,059 | 4,345 | |
| | 6 hours | 3,385 | 709 | 3,965 | − 8.8 |
| | 1 day | 3,385 | 952 | 3,810 | −12.3 |
| Co | Before | 3,010 | 842 | 3,731 | |
| | 3½ hours | 2,823 | 804 | 3,680 | − 1.4 |
| | 1 day | 2,910 | 784 | 3,758 | + 0.7 |
| Bo | Before | 3,560 | 922 | 4,300 | |
| | 5 hours | 3,600 | 1,002 | 4,300 | 0.0 |
| Beh | Before | 2,820 | 697 | 3,100 | |
| | 5 hours | 2,410 | 646 | 2,740 | −11.6 |
| | 1 day | 2,760 | 772 | 3,000 | − 3.2 |
| Bet | Before | 1,460 | 635 | 1,810 | |
| | 3 hours | 951 | 476 | 1,428 | −21.5 |
| | 1 day | 1,128 | 626 | 1,721 | − 4.9 |
| Ho | Before | 1,240 | 816 | 1,830 | |
| | 6 hours | 861 | 622 | 1,680 | − 8.2 |
| | 1 day | 880 | 828 | 1,811 | − 1.0 |
| | 2 days | 894 | 894 | 1,810 | |
| McC | Control | 2,605* | 1,010 | 3,620 | |
| | 5 hours | 2,710 | 1,003 | 3,770 | + 4.2 |
| | 1 day | 3,020 | 1,069 | 3,838 | + 6.3 |
| Da | Before | 1,058 | 370 | 1,320 | |
| | Before | 1,430 | 529 | 2,118 | |
| | 5 hours | 1,310 | 502 | 1,780 | + 3.6 |
| | 1 day | 1,486 | 530 | 1,911 | +11.2 |
| Mo | Before | 1,360 | 532 | 1,880 | |
| | Before | 1,400 | 635 | 1,800 | |
| | 4 hours | 1,100 | 481 | 1,380 | −25.0 |
| | 1 day | 1,149 | 521 | 1,680 | − 8.7 |

All volumes corrected to 37° C., prevailing barometric pressure and saturated with water vapor (BTPS).

* Control taken seven days after lipiodol.

mouthpiece and nose clip. When the oximeter baseline was found to be stable, turning of the stopcock at the end of a normal expiration allowed the subject to breathe from a reservoir of 100% oxygen and expire into the room. The instrumental dead space was 70 cc. Each period of oxygen breathing lasted from two to seven minutes and between determinations intervals of seven to ten minutes were allowed for the saturation to reach the room air baseline again. In each subject from three to nine measurements of saturation time were made and the results averaged.

The method of interpreting the records requires some comment. Fowler and Comroe (20) in measuring the saturation time used a single-channel Millikan oximeter which partially compensated for changes in total hemoglobin. The instrument was set at 96% saturation with the normal subject breathing room air. Because the moment of reaching maximum saturation when the subject breathed 100% oxygen was difficult to appraise accurately, Fowler and Comroe used as endpoint 0.5% saturation below the highest value. The instrumental lag was 0.07 seconds to reach 95% of the final value. This method of measurement requires the assumptions that identical deflections of the instrument in different individuals represent identical changes in saturation and also that all subjects were 96% saturated while breathing room air.

In our studies we measured the total deflection from room air to 100% oxygen and subtracted 10% of this deflection to obtain an arbitrary endpoint. The time in seconds between the first inspiration of 100% oxygen and this point is called T$_{90}$ or the saturation time.[5] The oximeter was thus used as a qualitative instrument only.

As a corollary of the above measurement, the lung-to-ear circulation time was also obtained from the records by measuring the interval from the first inspiration of 100% oxygen to the first upswing of the saturation curve recorded at the ear.

## RESULTS

### *Alveolar-arterial* Po$_2$ *gradient*

From our control studies on normal individuals an alveolar-arterial Po$_2$ gradient of less than 7.4 mm. Hg can be expected in 95% of normal individuals (mean plus 2 x standard deviation). Considering this value as the upper limit of normal it was found that out of 10 patients studied all but four had abnormally large gradients, ranging from 9.7 to 44.2 mm. Hg at rest before lipiodol. This was associated in all instances with a normal "effective" alveolar Po$_2$. This increase in gradient can be considered as the result of an abnormally large contribution of mixed venous blood to the systemic arterial blood. This large "effective venous admixture" might result either from the presence of a significant number of perfused but nonventilated alveoli or from impaired diffusion across a pathologically thickened alveolar membrane with or without adequate ventilation. Arterial oxygen saturation less than normal (93%) was found in the four cases with the greatest gradients. These data are summarized in Table II.

After the administration of lipiodol the Po$_2$ gradient increased by 6 mm. Hg or less in all but two instances. These changes and that occurring in Da (a *decrease* of 2.7 mm. Hg) are of questionable significance since they are close to the limit of experimental error. In only one patient (Ho) was a significant increase in $P_{O_2}$ gradient found (31.6) and this persisted until 48 hours after the oil was given. This 56 year old man had advanced bronchiectasis of the left lower lobe and lingula associated with considerable emphysema and fibrosis. The change in his arterial oxygen saturation (5.4%), although not the largest in the series, reduced it to a definitely anoxic level from a control value close to normal. This individual was the only one of the six in this series who eventually underwent pulmonary resection who did not survive the postoperative period. He died on his fourth postoperative day of respiratory insufficiency. The remaining patients survived operation without unusual respiratory symptoms. Mo, who appeared to be just as poor an operative risk, had only a minimal change in alveolar-arterial gradient after lipiodol although the reduction in his vital capacity was the largest in the series (460 cc.). Statistical analysis of the data as a whole, using Student's t-test indicates no significant change in alveolar-arterial $P_{O_2}$ gradient nor in absolute value of arterial $P_{O_2}$ after lipiodol administration.

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[5] Our data are nevertheless roughly comparable to those of Fowler and Comroe (20) for the following reasons. These authors noted a mean increase of 3.72% saturation. From this 0.5% was subtracted for the endpoint which thus occurred 3.22% above the room air level. This is 3.22/3.72 or 86% of the total deflection which compares quite closely with our endpoint (90% of total deflection).

From the above findings the conclusion can be drawn that the presence of deficient alveolar function *per se* does not necessarily predispose to further impairment by lipiodol, but when this does occur it may be an ominous sign.

As might be expected from the greater diffusibility of carbon dioxide, the data reveal no significant increase in arterial $P_{CO_2}$ following lipiodol except in one case (Beh). As this patient had no demonstrable lung pathology the finding is probably due to experimental error.

### *Arterial oxygen saturation*

Although some cases had a fall in saturation as large as 5% after lipiodol most values remained within the range of normal (*i.e.*, above 93%). Three patients (Bet, Mo and Ho) did develop a significant degree of desaturation after bronchography, but in the former two this was not associated with a significant change of arterial $P_{O_2}$ or of alveolar-arterial $P_{O_2}$ gradient. Although the change in oxygen saturation occurring within several hours after bronchography was found to be statistically significant, it should be pointed out that duplicate determinations may differ by as much as 2% saturation.[6]

### *Vital capacity*

Statistical analysis of the vital capacity data (Table III) indicates a significant reduction ($P < .05$) from the control value on the day the bronchograms were made (average reduction 7.6%) but not on the following day (average difference 1.5%). Individually two cases showed a slight increase in vital capacity after lipiodol, two showed minimal or no change and four had from 8–25% reduction within several hours with a return to within 9% or less of the control value by the following day. Only one case (Ha) showed a progressive decrease in vital capacity amounting to a maximum loss of 12.3%. This man, however, had no demonstrable pulmonary pathology and his cooperation was open to question.

### *Oximeter data*

The results of the studies of saturation time are shown in Table IV. In the four normal cases

TABLE IV

*Saturation time in normal subjects and patients before and after lipiodol*

| Normal subject | Saturation time | Patient | Saturation time | | | B – A |
|---|---|---|---|---|---|---|
| | | | A Before | B After 2–7 hrs. | C After 24 hrs. | |
| | *seconds* | | *seconds* | *seconds* | *seconds* | *seconds* |
| GB | 31.5 | Bu | 86.6 | 53.9 | 47.1* | –32.7 |
| GB | 26.4 | Ha | 22.1 | 19.7 | 18.0 | – 2.4 |
| AR | 22.5 | Bet | 31.0 | 32.3 | 51.5 | 1.3 |
| AR | 22.4 | Co | 44.5 | 44.6 | 38.9 | 0.1 |
| AR | 29.4 | Ho | 82.4 | 101.9 | 132.2<br>81.9† | 19.5 |
| AR | 28.4 | Bo | 23.8 | 19.2 | — | – 4.6 |
| RP | 24.6 | Da | 24.6 | — | — | — |
| WE | 24.0 | | | | | |
| Mean | 26.2 | | 45.0 | 45.3 | 57.5 | – 3.2 |
| S.D. | 3.4 | | 28.0 | 30.3 | 43.7 | |

Each value in this table represents the average of from two to five readings taken consecutively on the same day.
* Two days after lipiodol.
† Four days after lipiodol.

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[6] For this reason and because of the nearly horizontal course of the oxygen dissociation curve in the high range of oxygen saturation, derivation of oxygen tension from oxygen saturation in this range is not permissible and the direct measurement of tension becomes a more sensitive index of changes.

considerable variability was found, the greatest difference between readings at a single session in the same subject being 17.0 seconds. The mean normal saturation time was 26.2 (±3.4 seconds). This compares closely with the average of 15–30 seconds found by Elam and colleagues (17) with the same equipment and a mean of 25.0 found by Wood, Taylor and Knutsen (21) with their instrument. Using the Millikan oximeter Fowler and Comroe (20) found a mean of 52.0 seconds ± 18.6 and Douglas and Edholm (22) 58.4 ± 6.1.

In the six patients studied a mean saturation time before lipiodol of 45.0 seconds ± 28.0 was found. The reproducibility of the data was comparable to that in the normal subjects except in one case in which a difference of as much as 47.0 seconds was found between successive readings. The administration of lipiodol did not result in a consistent change in saturation time, the mean change amounting to − 3.2 seconds.

It should be emphasized that the saturation time is influenced by several mechanical and physiologic factors other than the transport of $O_2$ across the alveolar membrane. Among these are proper timing of the beginning of inspiration of $O_2$, efficiency of intrapulmonary mixing, circulation time from lung to ear and instrumental factors including changing ear lobe volume as the edema from histamine subsides. Inability to control these rigidly undoubtedly accounts for the poor reproducibility.

Measurements of the lung-to-ear circulation time was also obtained from the records. In normals a mean of 5.4 seconds ± 0.7 was found. The mean for the patients (6.6 seconds ± 2.0) was not significantly different. No prolongation of circulation time after lipiodol was observed. (Average several hours after bronchography 6.5 ± 1.1; one day afterwards 6.2 ± 1.7.)

## DISCUSSION

From our studies of the alveolar-arterial $P_{O_2}$ gradient the conclusion can be reached that in most instances lipiodol in the amounts commonly used does not increase the proportion of mixed venous blood reaching the arterial circulation ("effective venous admixture"). It, therefore, neither decreases alveolar ventilation nor does its presence on the alveolar surface form a significant barrier to diffusion of oxygen. Even in those cases with an abnormally high control gradient, lipiodol does not generally appear to add further to the abnormality. This applies both to patients with diffuse pulmonary disease (emphysema) as well as to those with more localized processes such as parenchymal infiltration or atelectasis. The pulmonary pathology of the one case in our series with a considerable increase in gradient following lipiodol was not fundamentally different from that found in the other patients, except perhaps in extent. For the purposes of evaluating the advisability of delaying operation after lipiodol administration, however, it should be noted that even in this individual the gradient returned to the control level within 48 hours. In addition, all abnormal values found on any of the tests in the remaining patients had returned to the pre-lipiodol level within 24 hours.

It must be realized that varying amounts of bronchial secretion may result in spontaneous fluctuations in the oxygen gradient. Such fluctuations have actually been demonstrated in some of the patients in whom more than one control study was done. Any comparison between studies before and after lipiodol should be considered in the light of this varying baseline.

In evaluating our data on vital capacity and subdivisions comparison with the work of Zavod (23) can be made. This author found in 50 patients an average loss of 15% of vital capacity within one hour of the administration of lipiodol. This loss decreased to 10% on the following day and had disappeared by the fourth day. It was more at the expense of expiratory reserve volume than of inspiratory capacity. A similar trend is found in our data although the losses of vital capacity are not as marked and not as consistent. We are reluctant to attach any importance to the immediate reduction in vital capacity since it may be due to inadequate cooperation of the patient in whom the presence of residual lipiodol often precipitates cough on maximal expiratory effort.

In summary, we may conclude that the fear of further impairment of alveolar function need not delay an intrapleural operation more than a few days after bronchography.

## CONCLUSIONS

1. A study has been made of the pulmonary function of 10 patients before and after bronchography by means of lipiodol.

2. In only one patient was the alveolar-arterial $Po_2$ gradient found to be definitely increased following the procedure, although five cases in addition to this one had an abnormally high gradient in the control study.

3. Arterial carbon dioxide tension was essentially uninfluenced by lipiodol.

4. The vital capacity was reduced by an average of 7.6% several hours after bronchography.

5. Measurement of the saturation time on changing the inspired gas from room air to 100% oxygen revealed no significant effect after lipiodol. Lung-to-ear circulation time was similarly unchanged.

6. All abnormal values returned to the control level within 24 hours except in one case in whom the pre-lipiodol values were not reached until 48 hours later.

7. It is concluded that the presence of lipiodol has no significant influence on the respiratory gas exchange except in occasional patients with advanced pulmonary pathology.

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# THE EFFECT OF INTRAVENOUS INFUSIONS OF PHYSIOLOGIC SALINE SOLUTION ON THE PULMONARY ARTERIAL AND PULMONARY CAPILLARY PRESSURE IN MAN [1]

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(Submitted for publication November 11, 1950; accepted, January 8, 1951)

In view of the possible role of an increased blood volume in the development of certain features of congestive heart failure, we have undertaken an investigation of experimentally induced changes in plasma volume. The present report deals with the effect of rapid intravenous infusions of physiologic saline solution on the pulmonary arterial and capillary pressure of normal human subjects.

## METHODS

Twelve adult men ranging in age from 17 to 64 years and convalescent from noncardiac ailments were studied. The subjects were in the post-absorptive state and in the recumbent position. The pulmonary artery was catheterized by the usual technique (1). An indwelling needle was inserted into a peripheral artery. Expired air was collected for three minutes in a Douglas bag. The oxygen content of the blood samples was determined by the method of Van Slyke and Neill (2) and of the air samples by the method of Haldane (3). The cardiac output was calculated utilizing the Fick principle.

The so-called pulmonary blood volume was determined by the dye dilution method of Stewart and Hamilton (4, 5) as modified by Ebert (6). An accurately measured amount of T-1824 was injected into the cardiac catheter and the arterial blood sampled for two-second periods for 22 seconds after the injection. A ten-minute arterial blood specimen was obtained for estimation of the initial general blood volume. Subsequent blood volumes were calculated from the hematocrit reading, assuming the red blood cell mass to remain constant. The mean pulmonary circulation time and the cardiac output were calculated from the dye time-concentration curve and in turn the pulmonary blood volume was derived.

The instantaneous and mean pulmonary arterial pressure curves were measured from a zero level 5 cm. below the sternal angle by a Sanborn electromanometer and registered with a simultaneous electrocardiographic lead on a Sanborn Polyviso recorder. The so-called pulmonary capillary pressure was measured by the method of Dexter (7). The heart rate was determined from the electrocardiogram.

After completion of these control observations, physiologic saline solution was injected into a peripheral vein at rates of from 77–146 cc. per minute in amounts of 950–1,000 cc. During this time the pulmonary arterial pressure was measured continuously or at frequent intervals. Toward the end of the infusion period the cardiac output and the pulmonary blood volume were again determined. The infusion was then discontinued and in most instances the cardiac output was determined a third time at varying subsequent intervals.

Measurements of the vital capacity were made on eight subjects at various times during the experimental period.

## RESULTS

In each of the 12 cases there was a rise in the pulmonary arterial pressure evident within one or two minutes and continuing to increase throughout the infusion period (Table I). The mean resting pulmonary arterial pressure of 7–15 mm. Hg (average 12 mm. Hg) rose to levels of 16–25 mm. Hg (average 20 mm. Hg), representing increases of from 43–150 per cent. Both the systolic and diastolic pressures participated in the rise although the pulse pressure often widened moderately. Within 15 to 60 minutes after the infusion the mean pulmonary arterial pressure had returned to or toward normal.

In four cases tabulated and in four other cases not tabulated here, the rapid infusion of saline solution caused an increase in the pulmonary capillary pressure proportional to that observed in the pulmonary artery. Percentagewise the increase was greater because of the low initial level of the capillary pressure.

Hemodilution at the end of the infusion was indicated by a fall in the hematocrit reading averaging 13 per cent and by an average increase in the general blood volume of 14 per cent. This repre-

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[1] This study was supported in part by a grant from the American Heart Association.

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TABLE I

*Hemodynamic effect of rapid intravenous infusion of physiologic saline solution*

| Patient | Age | Surface area | Remarks | Hematocrit reading | General blood volume | Pulmonary blood volume | Vital capacity | Pulmonary arterial pressure | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | S | D | Mean |
| | *(years)* | *(sq. m.)* | | | *(cc./sq. m.)* | *(cc./sq. m.)* | *(l./sq. m.)* | *(mm. Hg)* | *(mm. Hg)* | *(mm. Hg)* |
| 1 | 39 | 1.82 | Control | 42.4 | 2,430 | 650 | 2.19 | 15 | 2 | 7 |
| | | | 1,000 cc. in 9½ min. | 37.1 | 2,780 | 650 | 1.93 | 22 | 7 | 16 |
| | | | 38 min. after infusion | 40.4 | 2,550 | — | 2.16 | 16 | 3 | 10 |
| 2 | 64 | 1.60 | Control | 32.3 | 2,980 | — | — | 28 | 6 | 15 |
| | | | 1,000 cc. in 11 min. | 27.6 | 3,480 | 650 | — | 35 | 15 | 23 |
| | | | 60 min. after infusion | 29.2 | 3,290 | — | — | 28 | 8 | 17 |
| 3 | 31 | 1.68 | Control | 37.7 | 2,450 | 480 | 1.67 | 20 | 5 | 12 |
| | | | 1,000 cc. in 10 min. | 31.6 | 2,920 | 570 | 1.43 | 36 | 11 | 22 |
| 4 | 27 | 1.65 | Control | 40.0 | 2,590 | 420 | 1.61 | 22 | 3 | 10 |
| | | | 1,000 cc. in 11 min. | 34.3 | 3,020 | 440 | 1.64 | 38 | 16 | 25 |
| | | | 35 min. after infusion | 33.2 | 3,120 | — | 1.45 | 18 | 3 | 8 |
| 5 | 36 | 1.63 | Control | 34.7 | 2,470 | 660 | — | 17 | 5 | 8 |
| | | | 1,000 cc. in 11 min. | 28.4 | 3,020 | 800 | — | 25 | 10 | 16 |
| 6 | 17 | 1.65 | Control | 47.5 | 2,750 | 590 | 2.24 | 20 | 5 | 10 |
| | | | 1,000 cc. in 13 min. | 42.4 | 3,080 | 780 | 2.16 | 25 | 10 | 19 |
| | | | 15 min. after infusion | 44.3 | 2,950 | — | 2.27 | 20 | 5 | 10 |
| 7 | 42 | 1.71 | Control | 43.9 | 2,390 | 490 | 2.20 | 22 | 5 | 10 |
| | | | 1,000 cc. in 11 min. | 37.8 | 2,780 | 710 | 1.98 | — | — | 15 |
| | | | 30 min. after infusion | 40.2 | 2,610 | 620 | 2.22 | 19 | 5 | 10 |
| 8 | 34 | 1.95 | Control | 52.3 | 2,890 | 620 | 2.01 | 22 | 7 | 15 |
| | | | 950 cc. in 6½ min. | 45.2 | 3,280 | 500 | 2.05 | 35 | 15 | 23 |
| | | | 30 min. after infusion | 44.4 | 3,330 | — | — | 32 | 11 | 20 |
| 9 | 25 | 1.93 | Control | 44.1 | 2,620 | 490 | 1.80 | 20 | 5 | 11 |
| | | | 950 cc. in 7½ min. | 38.1 | 3,040 | 520 | 1.63 | 27 | 7 | 18 |
| 10 | 44 | 1.76 | Control | 45.7 | 3,200 | — | 2.35 | 17 | 6 | 11 |
| | | | 950 cc. in 8½ min. | 44.8 | 3,260 | — | 2.42 | 24 | 10 | 16 |
| | | | 30 min. after infusion | 43.1 | 3,390 | — | — | 23 | 10 | 15 |
| 11 | 21 | 1.71 | Control | 46.9 | 2,870 | 660 | — | 23 | 7 | 15 |
| | | | 900 cc. in 9 min. | 42.4 | 3,170 | 620 | — | 35 | 15 | 24 |
| | | | 40 min. after infusion | 44.9 | 2,990 | 560 | — | 25 | 7 | 15 |
| 12 | 49 | 1.83 | Control | 47.9 | — | 560 | — | 27 | 7 | 14 |
| | | | 900 cc. in 8½ min. | 42.0 | — | 690 | — | 33 | 12 | 20 |
| Averages | 36 | 1.74 | Control | 43.0 | 2,700 } p .001 | 560 } .2 > p > .1 | 2.01 | 21 | 5 | 12 } p .001 |
| | | | End of infusion | 37.6 | 3,080 | 630 | 1.91 | 30 | 12 | 20 |
| | | | After infusion | 40.0 | 3,030 | 590 | 2.03 | 23 | 7 | 13 |

TABLE I—*Continued*

| Patient | Age | Surface area | Remarks | Pulmonary capillary pressure | Mean circulation time | Pulse rate | Oxygen consumption | Arteriovenous oxygen difference | Cardiac index (Fick) | Cardiac index (Dye) |
|---|---|---|---|---|---|---|---|---|---|---|
| | *(years)* | *(sq. m.)* | | *(mm. Hg)* | *(sec.)* | *(beats/min.)* | *(cc./sq. m./min.)* | *(cc./100 cc.)* | *(l./sq. m./min.)* | *(l./sq. m./min.)* |
| 1 | 39 | 1.82 | Control | — | 15.3 | 60 | 178 | 4.3 | 4.2 | 2.6 |
| | | | 1,000 cc. in 9½ min. | — | 13.7 | 60 | 186 | 3.8 | 4.9 | 2.9 |
| | | | 38 min. after infusion | — | — | 60 | 187 | 4.2 | 4.5 | — |
| 2 | 64 | 1.60 | Control | — | 16.5 | 75 | 175 | 3.4 | 5.2 | — |
| | | | 1,000 cc. in 11 min. | — | 14.6 | 75 | 176 | 3.5 | 5.0 | 2.7 |
| | | | 60 min. after infusion | — | — | 75 | 149 | 4.5 | 3.3 | — |
| 3 | 31 | 1.68 | Control | — | 9.9 | 65 | 142 | 4.1 | 3.6 | 3.0 |
| | | | 1,000 cc. in 10 min. | — | 7.5 | 70 | 146 | 2.5 | 5.9 | 4.6 |
| 4 | 27 | 1.65 | Control | — | 8.9 | 100 | 164 | 3.3 | 5.0 | 2.8 |
| | | | 1,000 cc. in 11 min. | — | 7.7 | 100 | 147 | 2.9 | 5.2 | 3.5 |
| | | | 35 min. after infusion | — | — | 90 | 129 | 3.1 | 4.1 | — |
| 5 | 36 | 1.63 | Control | — | 11.4 | 95 | 150 | 4.3 | 3.5 | 3.5 |
| | | | 1,000 cc. in 11 min. | — | 12.1 | 85 | 172 | 3.2 | 5.3 | 4.0 |
| 6 | 17 | 1.65 | Control | 5 | 11.0 | 90 | 161 | 4.2 | 3.8 | 3.2 |
| | | | 1,000 cc. in 13 min. | 14 | 11.3 | 80 | 169 | 3.6 | 4.7 | 4.1 |
| | | | 15 min. after infusion | 5 | — | 70 | 168 | 4.0 | 4.7 | — |
| 7 | 42 | 1.71 | Control | 5 | 14.4 | 70 | 110 | 4.5 | 2.5 | 2.0 |
| | | | 1,000 cc. in 11 min. | 10 | 11.9 | 75 | 121 | 4.1 | 2.9 | 3.6 |
| | | | 30 min. after infusion | — | 14.5 | 75 | 132 | 4.0 | 3.3 | 2.6 |
| 8 | 34 | 1.95 | Control | 6 | 13.3 | 75 | 142 | 5.6 | 2.5 | 2.8 |
| | | | 950 cc. in 6½ min. | — | 11.4 | 70 | 149 | 4.8 | 3.1 | 2.6 |
| | | | 30 min. after infusion | — | — | 65 | 150 | 4.6 | 3.3 | — |
| 9 | 25 | 1.93 | Control | — | 10.8 | 75 | 130 | 3.5 | 3.7 | 2.7 |
| | | | 950 cc. in 7½ min. | — | 10.5 | 80 | 169 | 3.9 | 4.3 | 3.0 |
| 10 | 44 | 1.76 | Control | — | — | 75 | 166 | 5.9 | 2.7 | — |
| | | | 950 cc. in 8½ min. | — | 11.1 | 60 | 167 | 5.5 | 3.0 | — |
| | | | 30 min. after infusion | — | — | 65 | 162 | 5.9 | 2.8 | — |
| 11 | 21 | 1.71 | Control | 6 | 13.7 | 65 | 130 | 4.3 | 3.0 | 2.9 |
| | | | 900 cc. in 9 min. | 15 | 7.8 | 65 | 161 | 3.9 | 4.2 | 4.8 |
| | | | 40 min. after infusion | 8 | 10.6 | 65 | 134 | 4.6 | 2.9 | 3.2 |
| 12 | 49 | 1.83 | Control | 4 | 11.4 | 80 | 151 | 4.4 | 3.4 | 3.0 |
| | | | 900 cc. in 8½ min. | 12 | 12.9 | 80 | 154 | 4.3 | 3.6 | 3.2 |
| Averages | 36 | 1.74 | Control | — | 12.4} .2>p>.1 | 80 | 150} .2>p>.1 | 4.3} .7>p>.6 | 3.6} .05>p>.02 | 2.9} .02>p>.01 |
| | | | End of infusion | — | 11.0} | 80 | 160} | 3.8} | 4.3} | 3.6} |
| | | | After infusion | — | | 70 | 151 | 4.4 | 4.4 | — |

sented an increase in plasma volume of approximately 385 cc. per square meter in an average subject.

In 10 cases the pulmonary blood volume showed an average post-infusion increase of 12 per cent with variations from − 6 to + 45 per cent of the control value. Both before and after the infusion the pulmonary blood volume amounted to about 20 per cent of the general blood volume. Individually, measured increases in the pulmonary and the general blood volumes were not always proportional. Five of eight subjects showed a decrease in the vital capacity at the end of the infusion.

The response of the cardiac output to the infusion was variable. The minute oxygen consumption rose slightly after the infusion. The arteriovenous oxygen difference and the central circulation time in general were somewhat reduced by the infusion. In six cases there was a significant increase in the cardiac output after the infusion while six remained within the limits of experimental error. In six out of eight cases, the third cardiac output was the same as or below the control level.

The pulse rate remained remarkably constant during the control and rapid infusion periods. In four patients repeated direct arterial pressure measurements failed to demonstrate a significant change following the saline infusion. Distention of the peripheral veins was regularly observed. Flushing of the skin was not seen. None of the subjects complained of dyspnea.

## DISCUSSION

In every instance the pulmonary arterial and pulmonary capillary pressures were promptly and strikingly increased by the infusion of physiologic saline solution. A similar response has been observed following the transfusion of whole blood and the infusion of isotonic glucose and dextran solutions (8). Conversely, observations in this laboratory have demonstrated that a reduction of the effective circulating blood volume, caused by the application of venous-occluding tourniquets about the thighs, produces a lowering of the pulmonary arterial pressure (9). These data suggest that an abrupt, if not large, alteration in blood volume is the common denominator producing the observed pulmonary pressure changes.

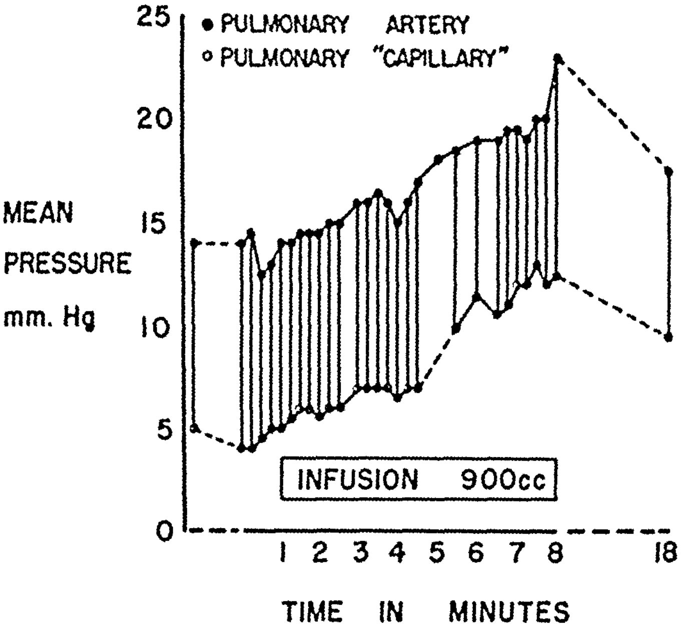

FIG. 1. SIMULTANEOUS MEASUREMENT OF PULMONARY ARTERIAL AND CAPILLARY PRESSURES DURING A RAPID SALINE INFUSION

There was no significant change in cardiac output.

On theoretical grounds the rise in pulmonary arterial pressure following an infusion might be the result of an increased cardiac output and therefore an increased pulmonary blood flow; of increased pulmonary resistance due to vasoconstriction within the lungs; or of an increased volume of blood contained within the pulmonary vascular bed.

The fact that the rise in pulmonary arterial pressure was the result of a rapid infusion suggested that the pressure elevation might be the result of increased flow through the pulmonary circuit. Although an increased cardiac output, and therefore an increased pulmonary flow, did occur in six instances, in the other six there was only an insignificant change in the cardiac output, yet the hypertension also resulted. In addition, there was no correlation between the magnitude of the increase in the pulmonary arterial pressure and the changes in cardiac output which did occur. This finding is not entirely surprising because previous observations on normal subjects during light exercise (10) and on patients during temporary occlusion of arteriovenous fistulas (11) have demonstrated that a considerable change in the cardiac output may occur without any alteration in the pulmonary arterial pressure.

As in the case of the pulmonary arterial pressure, there was an increase in the so-called pulmonary capillary pressure in all the patients observed. Although continuous and simultaneous pressure readings of this function and of the pulmonary arterial pressure were not usually technically possible, intermittent readings gave evidence that there was little or no change in the pressure relationships between the two areas. In one experiment two catheters were introduced, one measuring the pulmonary arterial and the other pulmonary capillary pressure. It was demonstrated that a saline infusion raised both pressures, the gradient between the two remaining constant (Figure 1). The evidence of an elevated pulmonary capillary pressure indicates that quite probably the pulmonary venous pressure is also increased. Indeed, it may be that the pressure measured by this technique actually represents the left atrial pressure rather than the pulmonary capillary pressure (12), for the contours often show typical a-c-v waves. Further support to the concept that intravenous infusions raise the venous as well as the arterial pressure in the pulmonary circuit has been obtained from animal experiments in which both pressures were simultaneously measured with catheters in the pulmonary artery and the pulmonary vein (13). With large infusions it was found that both pressures rise, and that the venous pressure eventually approaches the elevated pulmonary arterial pressure. These data, therefore, indicate that the intravenous infusions in our patients produced a rise in the pulmonary arterial, capillary and venous pressures without gross alteration in the pressure relationships between these three components of the pulmonary circuit.

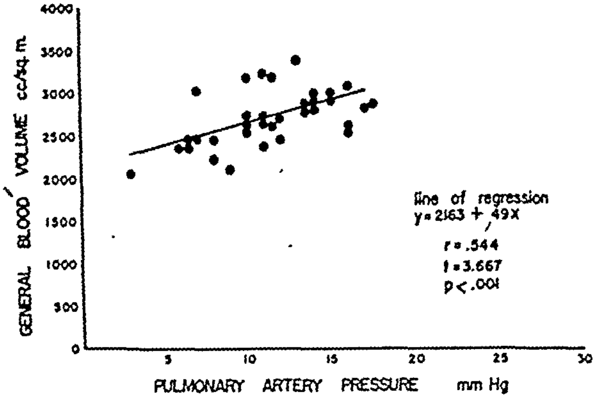

FIG. 2. THE RELATIONSHIP BETWEEN THE GENERAL BLOOD VOLUME AND THE PULMONARY ARTERIAL PRESSURE IN SUBJECTS WITH NORMAL CARDIOPULMONARY FUNCTION UNDER RELATIVELY BASAL CONDITIONS

TABLE II

*Observations on patients with normal cardiopulmonary functions under relatively basal conditions*

| Patient | Age | Surface area | Hematocrit reading | Blood volume | Pulmonary arterial pressure | Cardiac index (Fick) |
|---|---|---|---|---|---|---|
| | *(years)* | *(sq. m.)* | | *(cc./ sq. m.)* | *(mm. Hg)* | *(l./min./ sq. m.)* |
| 1 | 39 | 1.55 | 38.4 | 2,710 | 11.0 | 4.5 |
| 2 | 33 | 1.59 | 41.6 | 3,080 | 16.0 | 6.2 |
| 3 | 59 | 1.62 | 28.1 | 2,360 | 6.0 | 5.3 |
| 4 | 43 | 1.48 | 34.0 | 2,700 | 12.0 | 2.4 |
| 5 | 35 | 1.76 | 49.0 | 3,030 | 7.0 | 3.3 |
| 6* | 17 | 1.65 | 47.5 | 2,750 | 10.0 | 3.8 |
| 7 | 53 | 1.94 | 44.0 | 3,230 | 11.0 | 3.3 |
| 8 | 24 | 1.66 | 41.7 | 2,980 | 14.0 | 3.6 |
| 9 | 20 | 2.04 | 38.1 | 2,850 | 14.0 | 4.7 |
| 10 | 18 | 1.82 | 48.0 | 2,630 | 10.0 | 3.4 |
| 11 | 24 | 1.96 | 31.6 | 2,400 | 6.5 | 3.0 |
| 12 | 56 | 1.68 | 39.0 | 2,120 | 9.0 | 1.7 |
| 13* | 25 | 1.93 | 44.1 | 2,620 | 11.5 | 3.7 |
| 14* | 34 | 1.95 | 52.3 | 2,830 | 17.0 | 2.8 |
| 15* | 42 | 1.71 | 43.9 | 2,390 | 11.0 | 2.5 |
| 16 | 42 | 1.96 | 44.9 | 2,860 | 17.5 | 4.6 |
| 17* | 64 | 1.60 | 32.3 | 2,980 | 15.0 | 5.2 |
| 18* | 31 | 1.68 | 37.7 | 2,450 | 12.0 | 3.6 |
| 19 | 21 | 1.88 | 43.8 | 2,970 | 14.0 | 3.8 |
| 20* | 27 | 1.65 | 40.0 | 2,590 | 10.0 | 5.0 |
| 21 | 39 | 1.85 | 36.5 | 2,050 | 3.0 | 2.4 |
| 22 | 63 | 1.61 | 47.5 | 2,940 | 15.0 | 4.0 |
| 23 | 49 | 1.91 | 36.8 | 2,610 | 16.0 | 3.6 |
| 24* | 39 | 1.82 | 42.4 | 2,430 | 6.5 | 4.2 |
| 25 | 59 | 1.77 | 43.9 | 3,380 | 13.0 | |
| 26 | 31 | 1.30 | 27.0 | 2,710 | 11.0 | 5.5 |
| 27 | 34 | 1.87 | 32.9 | 2,250 | 8.0 | 4.8 |
| 28 | 44 | 1.40 | 34.0 | 2,560 | 16.0 | 2.7 |
| 29* | 44 | 1.76 | 45.7 | 3,200 | 11.5 | 2.7 |
| 30 | 34 | 1.51 | 38.5 | 2,470 | 8.0 | 5.1 |
| 31* | 21 | 1.71 | 46.9 | 2,870 | 13.5 | 3.0 |
| 32* | 36 | 1.63 | 34.7 | 2,470 | 7.0 | 3.5 |
| 33 | 47 | 1.82 | 39.2 | 3,180 | 10.0 | 3.6 |
| 34 | 28 | 1.66 | 48.5 | 2,820 | 13.5 | 4.3 |
| Mean | 37.5 | 1.73 | 40.4 | 2,720 | 11.4 | 3.8 |
| S.D. | | | | ±316 | ±3.49 | ±1.03 |

* Denotes patients receiving saline infusions and included in Table I.

The maintenance of essentially normal pressure gradients throughout the pulmonary circuit in subjects receiving rapid infusions is considered as evidence against the occurrence of pulmonary arteriolar constriction as the cause of the pressure changes in these subjects. Had arteriolar constriction been the cause of the increased pulmonary arterial pressure, little or no increase in the pulmonary capillary pressure would have been observed. Further evidence against vasoconstriction was obtained by examination of the contour of the pulmonary arterial pressure tracings which revealed no indication of decreased "run-off" during diastole.

If increased flow and increased resistance due to arteriolar constriction may be eliminated as the fundamental mechanisms producing the pulmonary hypertension, it becomes necessary to explore the possibility of an increased volume of blood in the lungs.

First, the possible relationship of the pulmonary arterial pressure to the volume of blood in the pulmonary circuit under relatively basal conditions was investigated. A study of the so-called "pulmonary blood volume" failed to show good correlation with the pulmonary pressures. On the other hand, the relationship of the pulmonary arterial pressure to the general blood volume revealed a striking correlation. This is demonstrated in Figure 2, where in addition to the observations made in this study, there are included for further statistical validation 22 points derived from studies by the same methods on additional patients with normal cardiopulmonary function made under relatively basal conditions (Table II). A linear relationship of good statistical significance is demonstrated. If the results obtained on the effect of saline infusions are plotted in a similar way, they will be seen to follow closely the line of regression derived from the observations on normal subjects under basal conditions (Figure 3). Although the line of regression is represented as straight, it is very possible that in actuality it is curved and follows more nearly the pressure-volume relationship shown by elastic structures or, more particularly, by isolated segments of veins (14, 15). Apparently, however, under the conditions of our studies, the relationship between the blood volume and the pulmonary arterial pressure is nearly linear. Infusion of one liter of saline solution in our human subjects, however, gave no evidence of the disproportionately large rise in pulmonary pressures such as reported in the open-chested dog by Opdyke and associates (16).

The studies reported here indicate that the ratio between the quantities of blood contained in the pulmonary and the systemic circuits is not altered by a rapid expansion of the general blood volume. Yet, because of the pressure-volume relationship present in the pulmonary venous-left atrial segment, this small increase in blood content produced a considerable increase in pulmonary pressures. Similar evidence in the opposite direction is available from the study of blood loss in normal human subjects (8). If there were disproportionate addition to or loss from the pulmonary circulation, or if pulmonary vasomotor activity were stimulated, the relationship demonstrated in Figure 2 would not hold and the plotted

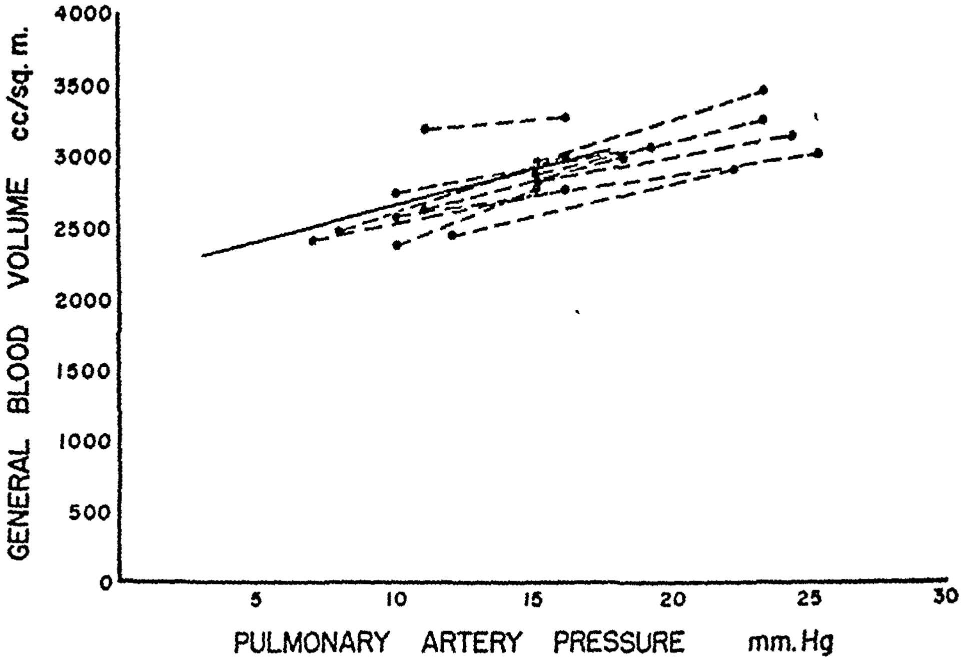

Fig. 3. The Relationship of the General Blood Volume and the Pulmonary Arterial Pressure in Normal Subjects Receiving Saline Infusions

In each case the pulmonary arterial pressure rises. The line of regression is that obtained from Figure 2.

results would not fall on the line of regression. During hypoxia in normal subjects and in certain pathologic situations such a disproportion does occur (8). These pressure relationships are evidence against, but do not eliminate the unlikely possibility that our infusions produced acute cardiac failure.

The reason for the absence of a good correlation between the observed pulmonary arterial pressure and the measured pulmonary blood volume in our studies is not apparent. The mean values show the expected directional trend but the individual values appear to be somewhat erratic. It is felt probable that changes in the pulmonary volume of the order of 50–100 cc., as induced in many of these experiments, are not sufficiently beyond the range of error in the measurement itself (8).

Insofar as one can measure the filling pressure from observations made at one point, it is interesting to note that despite the evidence of an increase in the pulmonary venous, and therefore the left heart filling pressure, there was not a consistent increase in the left ventricular output. These data are compatible with the concept that alterations in the filling pressure are not a major factor in producing changes in the cardiac output in the human being (17).

It has been demonstrated previously that intravenous infusions similar to those utilized here produce an increase in the right ventricular, right atrial, central venous and peripheral venous pressures (8, 18). Although simultaneous readings of these pressures and those in the pulmonary circuit are not available, the evidence indicates that the rise produced is essentially the same throughout the venous and pulmonary circuit. The normal pressure gradients appear to be maintained thereby from peripheral vein to left atrium, but at a higher level following the infusion.

In the present study the pulse rate remained remarkably constant from the control through the infusion period. This failure to observe the Bainbridge reflex in the normal human has been observed previously in this laboratory (18).

It is difficult to assess the relationship of the pulmonary hypertension produced in these studies to that occurring in patients with cardiac disease. These experiments, as well as other recently reported observations (19, 20), demonstrate the occurrence of elevations in the pulmonary capillary pressure of such magnitude as adequately to account for the production of pulmonary edema without implication of changes in capillary permeability. Further studies are under way on the effect of manipulations similar to those described here on the pulmonary hemodynamics of patients with cardiac and pulmonary disease.

## SUMMARY

In the normal human subject the pulmonary arterial and the pulmonary capillary pressure can be significantly increased by the rapid intravenous infusion of physiologic saline solution. The pulmonary and the general blood volumes increase proportionately after this maneuver. The cardiac output after a rapid infusion may or may not increase.

There is a linear correlation between the resting pulmonary arterial pressure and the general blood volume in normal subjects but this relationship is not apparent in the case of the pulmonary blood volume as measured by the dye technique. The same relationship continues after an increase in the blood volume with saline solution. It is suggested that the changes induced by a rapid infusion can be explained most adequately on the basis of an abrupt, but not disproportionate, increase in the amount of blood contained in the relatively indistensible pulmonary circuit.

## ACKNOWLEDGMENT

This work was carried out with the technical assistance of Misses Eloise Cavin, Mary Upshaw, Voncile Williams, Elizabeth Kelley, Mrs. Fransetta Latimer, and Mrs. Louise Thompson.

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# COMPARISON OF INTRACARDIAC AND INTRAVASCULAR TEMPERATURES WITH RECTAL TEMPERATURES IN MAN[1, 2]

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(Submitted for publication September 28, 1950; accepted, January 22, 1951)

A study of intracardiac and intravascular temperatures was undertaken with the thought that such measurements might provide further information concerning the mechanisms which normally maintain body temperature within its narrow limits and also that they might help to explain the deviations from normal temperature in certain disease states. Whereas the temperature of the peripheral tissues may vary widely, homeothermic man maintains the temperature of the deep tissues, or of a critical deep tissue, fairly constant under widely varying conditions of heat loss and heat gain (2). The identity of the critical deep tissue is not completely understood but a considerable body of evidence places it in the diencephalon and in normal man small variations in its temperature are thought to initiate compensating mechanisms which return the temperature of the body to the homeothermic norm. Generally, rectal temperature has been considered to indicate deep tissue, and critical tissue, temperatures but often rectal temperature appears to be a lagging and unsatisfactory index; witness the onset, when the body is heated, of such compensating mechanisms as peripheral vasodilatation and sweating before a rise in rectal temperature has occurred; and conversely, in subjects who are cooled, the presence of vasoconstriction and pilomotor activity before a fall in rectal temperature has taken place. It seemed desirable, therefore, to look for some other index of critical deep tissue temperature.

Considerations of the mixing of blood in the left ventricle, probably in proportion to the volume flow and temperature of the blood from all organs, suggested the temperature of left ventricular blood as representative of deep tissue temperature. The difficulties in such a measurement, together with the likelihood that no significant change in temperature occurs during the transit of blood through the aorta, lead to the substitution of the temperature of the blood high in the femoral artery for left ventricular temperature. The observation (3) that such femoral arterial temperatures consistently fell below simultaneously recorded rectal temperatures, and that rectal temperature lagged behind changes in temperature of femoral arterial blood during changing body temperature, prompted the measurement of more "central" blood temperatures.

It was the purpose of the present study to determine the intracardiac and deep intravascular temperatures in afebrile subjects and thus establish a baseline of values with which future determinations, obtained during abnormal and changing body temperatures, could be compared.

## METHODS

Observations were made on 24 afebrile hospitalized subjects, usually several hours after breakfast and occasionally after an overnight fast. Most of the determinations were made at ambient temperatures between 77° F. and 84° F. and with the subjects nude, except for a loin cloth. During the few observations made at lower room temperatures the subjects were covered with one sheet.

All temperatures were measured potentiometrically with naked-junction, copper-constantan thermocouples and a Leeds-Northrup, Type K2, potentiometer. The temperatures in the chambers of the right heart and in the deep vessels leading to the right heart were measured by a thermocouple (copper wire gauge 30, constantan wire gauge 30) threaded to the tip of a standard, 8F, intracardiac catheter. This was a roving thermocouple, introduced in varying succession by the technique of cardiac catheterization (4), and under fluoroscopic control, into the right heart and connecting vessels. In determining a temperature with this thermocouple, it was not moved to a new location until three successive read-

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[1] Read in part at the Thirty-third Annual Meeting of the American Physiological Society, Detroit, Michigan, April 21, 1949 (1).

[2] This study was supported by a grant from the Baruch Committee on Physical Medicine.

ings, made over a period of five to 10 minutes, checked each other.

All other thermocouples were kept stationary after they were positioned properly. Femoral arterial and femoral venous blood temperatures were obtained by indwelling, plastic-sheathed, thermocouples (copper wire gauge 42, constantan wire gauge 30) threaded through Cournand needles previously inserted into the corresponding vessels high in the groin (3, 5). The thermal junctions of these thermocouples extended in the lumen of the vessels for a distance of 5 to 6 centimeters beyond the needle tips and probably reached the junction of the femoral with the iliac vessels. Rectal temperature, 3 inches above the anal orifice, was determined by a single thermal junction (copper wire gauge 30, constantan wire gauge 30) presenting on the lateral wall of a suppository-shaped, hard-plastic applicator. Subcutaneous temperatures immediately beneath the skin, and intramuscular temperatures at a depth of 1½ inches below the skin surface, were determined in the lateral aspect of the thigh by means of gauge 22 needle thermocouples (copper wire gauge 42, constantan wire gauge 30). Because of their small masses all thermocouples had rapid response times, usually 95% of full deflection in 10 to 15 seconds. At the close of each experiment the calibration factor of each thermocouple was determined for that experiment.

## RESULTS

The data for a typical observation period are plotted in Figure 1, which shows the relationships of the various intracardiac and intravascular temperatures to each other, and to rectal temperature. These relationships are shown more definitively in Table I and in Figure 2, wherein the data for all of the observations are analyzed with reference to the rectal temperature at the time. The data are consistent and indicate several thermal gradients.

The temperatures in the right heart (atrium and ventricle) and pulmonary artery (main stem and

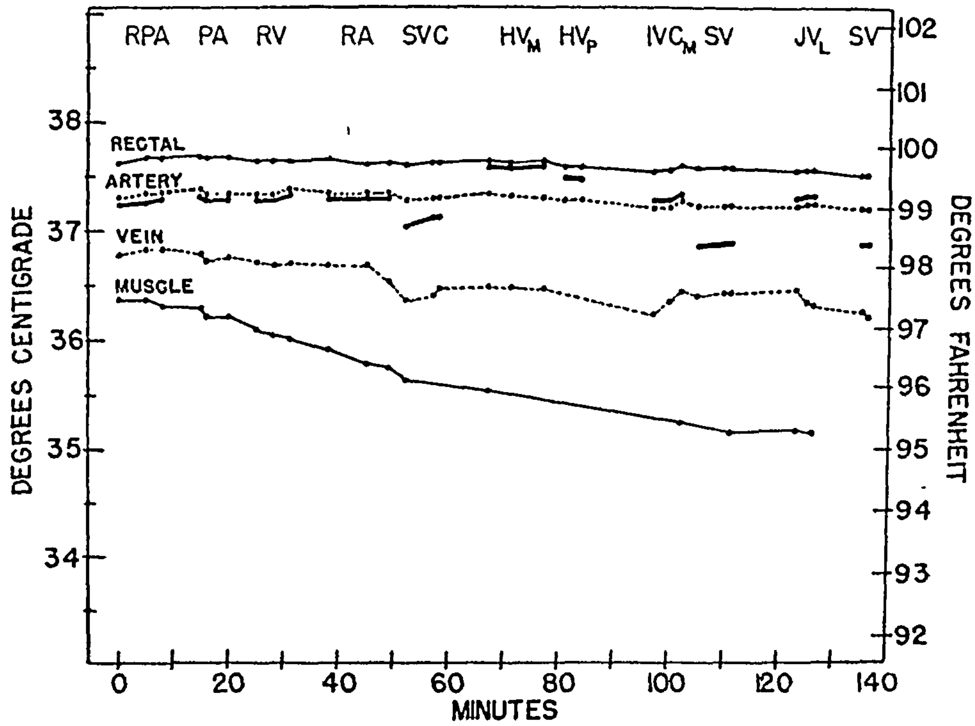

FIG. 1. OBSERVATIONS FROM A TYPICAL EXPERIMENT SHOWING THE RELATIONSHIPS OF INTRACARDIAC AND INTRAVASCULAR TEMPERATURES TO EACH OTHER AND TO RECTAL TEMPERATURES (AFEBRILE SUBJECT)

The lines from above downward indicate in turn, rectal temperature, femoral arterial temperature, femoral venous temperature and intramuscular (thigh) temperature. The heavy, disconnected, solid bars indicate the temperatures obtained with the catheter thermocouple in the locations indicated directly above by the letters at the top of the graph. In this and subsequent figures these letters indicate: RPA, right pulmonary artery; PA, main stem of pulmonary artery; RV, right ventricle; RA, right atrium; SVC, superior vena cava; SV and SC subclavian vein; $J_L$ and $JV_L$, jugular vein, low; $J_h$ and $JV_h$, jugular vein, high; $IVC_L$, inferior vena cava, low; $IVC_m$, inferior vena cava, mid-portion; $IVC_h$, inferior vena cava, high; $H_p$ and $HV_p$, hepatic vein near vena cava; $HV_m$, hepatic vein, mid-liver; $H_d$ and $HV_d$, hepatic vein deep in the liver; AV, axillary vein.

TABLE I

*Analysis of the deviations of intracardiac and intravascular temperatures from rectal temperature in afebrile subjects*

| Location of catheter thermocouple | Number of patients | Difference between "catheter" temperature and rectal temperature* | | | |
|---|---|---|---|---|---|
| | | Mean ° C. | Standard deviation ° C. | Fisher t value | Probability† P |
| Right Pulmonary Artery (RPA) | 16 | −0.27 | 0.17 | 6.24 | 0.01 |
| Pulmonary Artery Main Stem (PA) | 13 | −0.26 | 0.15 | 6.19 | 0.01 |
| Right Ventricle (RV) | 17 | −0.23 | 0.14 | 6.57 | 0.01 |
| Right Atrium (RA) | 24 | −0.26 | 0.13 | 9.96 | 0.01 |
| Superior Vena Cava (SVC) | 19 | −0.35 | 0.16 | 9.01 | 0.01 |
| Subclavian Vein (SV) | 21 | −0.58 | 0.22 | 11.55 | 0.01 |
| Jugular Vein, low ($J_L$) | 15 | −0.22 | 0.15 | 5.51 | 0.01 |
| Jugular Vein, high ($J_h$) | 4 | −0.01 | 0.04 | 0.22 | 0.80 |
| Inferior Vena Cava, low ($IVC_L$) | 7 | −0.26 | 0.22 | 2.84 | 0.02 |
| Inferior Vena Cava, high ($IVC_h$) | 10 | −0.22 | 0.18 | 3.63 | 0.01 |
| Hepatic Vein, near Vena Cava ($H_p$) | 6 | −0.09 | 0.10 | 1.88 | 0.10 |
| Hepatic Vein, deep in liver ($H_d$) | 6 | −0.03 | 0.04 | 1.84 | 0.10 |
| Femoral Artery (FA) | 22 | −0.22 | 0.15 | 6.66 | 0.01 |

* Minus indicates catheter temperature less than rectal temperature.

† Due to the small size of the samples, P values are not expressed smaller than 0.01 even though the P often calculated to a considerably smaller value.

right branch) were practically identical, essentially equal to femoral arterial temperature, and less than rectal temperature by an average of 0.25° C. (0.45° F.). Analysis shows that this difference between right heart and rectal temperature, though small and with a sizable standard deviation, is nonetheless statistically significant (Table I) because the intracardiac temperatures so consistently fell below rectal temperature (Figure 2).

Progressively lower temperatures were recorded as the thermocouple was moved from the right atrium into the superior vena cava and into the subclavian vein, where the temperature averaged 0.6° C. (1.1° F.) below rectal temperature (Figure 2). Statistically, the temperatures in both of these vessels are significantly below rectal temperature (Table I). In the inferior vena cava there were inconstant variations from right atrial temperature but on the average the temperature at all levels was approximately equal to that in the right atrium (Table I, Figure 2).

Two sites contributing blood of higher temperature to the circulation were noted, the brain and the liver. As the tip of the thermocouple passed from the subclavian vein into the common jugular vein, even for a distance of only a few centimeters, the blood temperature rose sharply from a relatively low value to a value equal to right heart temperature ($JV_L$, Figure 1). High in the jugular vein, approximately in the jugular bulb, the temperature was still higher, well above right heart temperature and equal to rectal temperature (Table I, Figure 2). Similarly the temperature of hepatic vein blood exceeded that of the inferior vena cava and equalled rectal temperature (Table I, Figure 2). The observations on hepatic and jugular blood temperatures were few in number but analysis suggests that these temperatures are probably the same as rectal temperature (Table I).

In brief, rectal temperature was slightly higher than the intracardiac and deep intravascular temperatures here measured, except that high in the jugular vein and deep in the hepatic vein the temperatures equalled rectal temperature.

In 20 afebrile subjects the temperature in the femoral artery was found to be similar to that in the right heart (Table II, Figure 3). Moreover, femoral arterial temperature was essentially the same as the temperature in most of the deep vessels. However, in the hepatic vein, and high in the jugular vein, the temperature was higher than femoral artery temperature and in the superior vena cava and subclavian vein the temperature was lower (Table II, Figure 3).

The differences between rectal and right heart temperatures were so small in afebrile subjects that for practical purposes the two temperatures may be considered to be the same. The few preliminary observations thus far made indicate that such a similarity may not be the case when body

TABLE II

*Analysis of the deviations of intravascular and intracardiac temperatures from femoral arterial temperature in afebrile subjects*

| Location of catheter thermocouple | Number of patients | Difference between "catheter" temperature and femoral artery temperature* | | | |
|---|---|---|---|---|---|
| | | Mean °C. | Standard deviation °C. | Fisher t value | Probability† P |
| Pulmonary Artery Main Stem (PA) | 16 | −0.03 | 0.07 | 1.36 | 0.2 |
| Right Ventricle (RV) | 17 | −0.01 | 0.06 | 0.46 | 0.6 |
| Right Atrium (RA) | 20 | −0.03 | 0.07 | 1.84 | 0.1 |
| Superior Vena Cava (SVC) | 17 | −0.11 | 0.08 | 5.83 | 0.01 |
| Subclavian Vein (SV) | 18 | −0.36 | 0.16 | 9.15 | 0.01 |
| Jugular Vein, low ($J_L$) | 11 | +0.02 | 0.12 | 0.62 | 0.5 |
| Jugular Vein, high ($J_h$) | 4 | +0.15 | 0.21 | 1.26 | 0.3 |
| Inferior Vena Cava, low ($IVC_L$) | 4 | −0.08 | 0.17 | 0.85 | 0.5 |
| Inferior Vena Cava, high ($IVC_h$) | 9 | +0.03 | 0.13 | 0.58 | 0.6 |
| Hepatic Vein, proximal ($H_p$) | 3 | +0.11 | 0.10 | 1.56 | 0.3 |
| Hepatic Vein, deep ($H_d$) | 4 | +0.26 | 0.05 | 9.37 | 0.01 |

* Plus indicates catheter temperature higher than rectal temperature; minus, catheter temperature less than rectal temperature.

† See footnote, Table I.

temperature departs from the homeothermic norm. The data plotted in Figure 4 were obtained in a febrile patient with slowly resolving pneumonia and show a very sizable difference (0.8° C., 1.4° F.) between rectal and right heart temperatures. Furthermore, in five febrile patients with rectal temperatures ranging from 38.2° C. to 40.0° C. (100.7° F. to 104.0° F.) the differences between right heart and rectal temperatures were 0.2°, 0.4°, 0.6°, 0.7° and 0.8° C. Right heart temperature

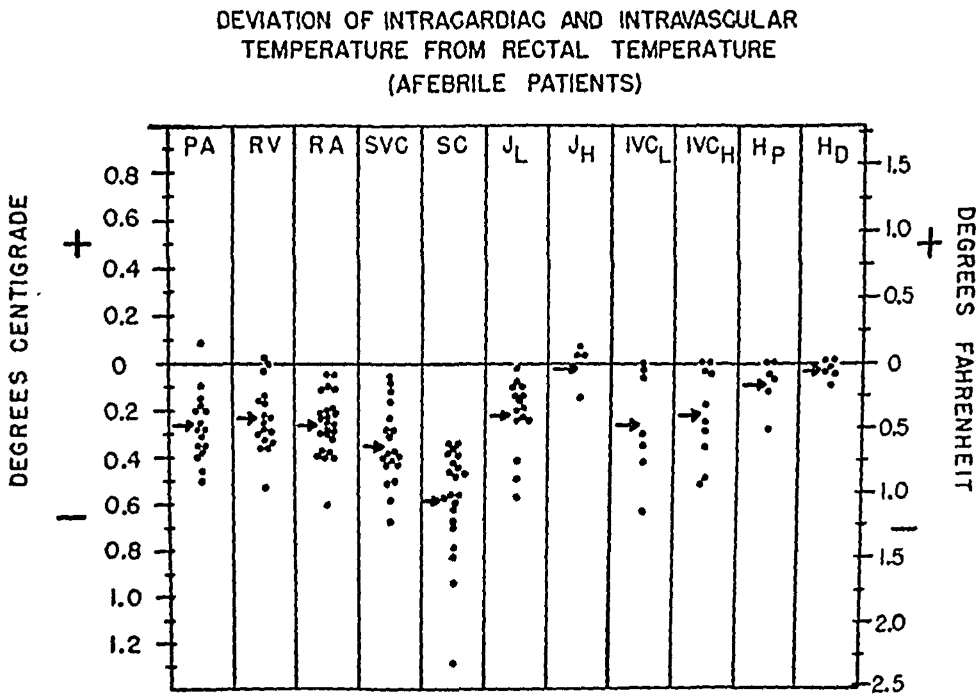

FIG. 2. Intracardiac and Intravascular Temperatures Plotted with Reference to the Simultaneously Obtained Rectal Temperature as a Bas (Afebrile Subjects)

Each dot represents a comparison in one subject between rectal temperature an the intravascular temperature indicated by the letters at the head of the respectiv columns. Each heavy horizontal arrow represents the average of the correspondin group of dots.

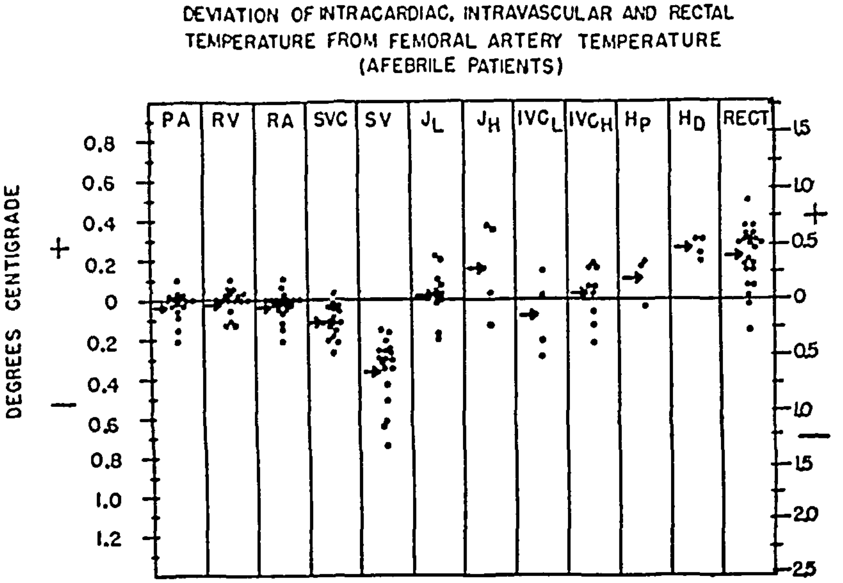

Fig. 3. Intracardiac, Intravascular and Rectal Temperatures Plotted with Reference to the Simultaneously Obtained Femoral Arterial Temperature as a Base (Afebrile Subjects)

Details as in Figure 2.

was always less than rectal temperature and the difference was greater in the patients with the higher fevers. In all five patients the relationships of the various intravascular temperatures to each other and to rectal temperature were the same as in afebrile subjects, and again, femoral arterial temperature was the same as right heart temperature.

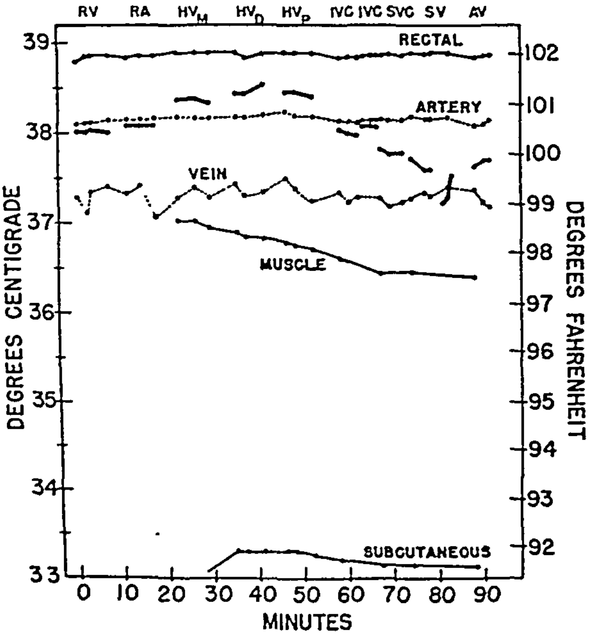

Fig. 4. Relationship of Intracardiac and Intravascular Temperatures to Each Other and to Rectal Temperature in a Febrile Patient with Slowly Resolving Pneumonia

Details as in Figure 1.

## DISCUSSION

The data indicate a small, but consistent, gradient of increasing temperature in the larger veins as they approach the heart. The gradient is steeper in the thorax than in the abdomen where, at times, it is not demonstrable. Sharp rises in temperature above these gradients occur within veins which drain organs considered to have increased metabolic heat production. The liver and brain are shown to be such organs.

It was not anticipated that femoral arterial temperature would be the same as right heart temperature. When previous observations (3) showed that femoral arterial temperature fell below rectal temperature, the supposition was held that right heart temperature probably equalled, or exceeded, rectal temperature and that the lower femoral arterial temperature was due to the cooling of blood within the lungs (assuming again that blood does not change in temperature during transit through the aorta). Since right heart and femoral arterial temperatures are essentially the same, it follows that heat loss from the lungs is *relatively* so small with respect to the large volume of blood which flows through the lungs that the temperature of the blood is not altered by an amount which can be determined by the methods here used.

Since the temperature of right heart blood was consistently less than rectal temperature, and since this difference was small, a systematic variation in the thermocouples was carefully looked for, but excluded. The consistency of the temperature pattern and the statistical analysis lead us to conclude that the difference between right heart and rectal temperature, though small, is a real one. Since the measurements were not made at controlled ambient conditions the significance and constancy of this small difference may be questioned, for it is well known that the temperatures of various portions of the body may vary considerably at different environmental temperatures and humidities. In this regard attention is called to the relatively narrow range (7° F.) of the ambient temperatures, all of which were in a zone comfortable for the nude subject. Furthermore, regardless of the variations in temperature and humidity within this range the relationship of the rectal and intracardiac temperatures was always the same, with respect to both the direction and the degree of difference.

It seems reasonable that there should be a difference between rectal and right heart temperatures since one gives the temperature of a single organ whereas the other is influenced by thermal contributions from all organs. It seems reasonable, too, that femoral arterial temperature should be similar to intracardiac temperature, since both determinations are measurements of mixed blood temperature. Furthermore, differences between intracardiac, intravascular and rectal temperatures of the magnitude and direction here reported have been demonstrated in animals; originally by Claude Bernard (6), who introduced thermometers into the heart and various vessels, and more recently by Horvath, Rubin and Foltz (7) who used intravascular thermocouples introduced under fluoroscopic control.

Although the differences between right heart temperature and rectal temperature were not sufficiently large to be of practical importance in afebrile subjects, these differences assume significance in two respects; in febrile patients and in considerations of the physiology of thermal homeostasis. In preliminary observations on febrile patients, right heart temperature at times fell so far below rectal temperature that the differences between the two cannot be dismissed. Further studies of thermal gradients within the vascular tree during fever may serve to clarify the factors responsible for maintaining the febrile state.

In regard to thermal homeostasis, attention has focussed on two temperatures; the average deep tissue temperature and the temperature of that "critical" tissue, believed to be located in the hypothalamus, which triggers the thermoregulating mechanisms which return body temperature to the homeothermic norm. Present concepts generally hold that both of these temperatures are measured by rectal temperature. This supposition appears open to question. The observations here reported confirm that variations in temperature exist even deep within the body core and this precludes the temperature of a single organ as the measure of *average* deep tissue temperature. An integrating measurement or a calculated average value seems more likely to indicate this temperature. Furthermore, evidence is not at hand to prove that the temperature of the rectum is the same as that of the critical tissue, particularly during conditions of changing body temperature.

Intracardiac temperature, and its equivalent femoral arterial temperature, appears to represent a "vascular" integration of the various individual temperatures to give an average blood temperature. This temperature seems to approach *average* deep tissue temperature more closely than any other single measurement but, because of the thermal contributions from the peripheral tissues, it may not be the same as average deep tissue temperature. Since the critical tissue temperature is the temperature of an organ, it is not likely that it would be indicated by an integrated temperature such as intracardiac temperature. Nevertheless, intracardiac and femoral arterial temperatures should have considerable significance in thermal homeostasis since the temperature of the blood as it enters, and affects, the critical tissue in the hypothalamus is more apt to be indicated by these temperatures than by any other measurement now made. Only the temperature of the blood in the afferent artery to the critical tissue would have greater significance and this is a measurement not now available.

The above considerations, based on the data presented, are suggested for the afebrile subject in thermal equilibrium. It remains for future study to determine whether the relationships between intracardiac, intravascular and rectal temperatures here reported, and the considerations drawn therefrom, also hold under the more critical conditions of changing body temperature, when thermoregulatory mechanisms are actually called into action.

## SUMMARY

1. Measurements of the temperature in the right heart, in the deep vessels leading to the right heart, in the femoral artery and in the rectum of 24 afebrile subjects showed:

(*a*) there is a gradient of increasing temperature in the larger veins as they approach the heart,

(*b*) temperatures within the right heart and pulmonary artery are equal, and the same as the temperature high in the femoral artery,

(*c*) rectal temperature exceeds intracardiac and deep intravascular temperatures by a small but significant amount,

(*d*) temperatures in the veins draining the liver and brain are higher than the temperatures in the veins into which they drain, and equal rectal temperature.

2. The differences between rectal and intracardiac temperatures are not of such magnitude as to be of clinical importance in afebrile subjects.

3. Preliminary observations suggest that during fever rectal temperature may exceed intracardiac temperature by a significant amount, up to 0.8° C. in one patient.

4. The data suggest a review of those concepts of thermal homeostasis which hold that rectal temperature represents either the average deep tissue temperature, or the temperature of the critical tissue which initiates thermoregulating mechanisms.

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# THE IMMUNOPHYSIOLOGY OF SERUM SICKNESS. ALTERATIONS IN THE BLOOD VOLUME AND THIOCYANATE SPACE IN RELATION TO THE DEVELOPMENT OF HUMORAL ANTIBODIES IN THE RABBIT[1]

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(Submitted for publication November 22, 1950; accepted, January 22, 1951)

The development of edema during the course of acute infectious diseases has been observed in patients for many years. The retention of water and salt during the course of untreated pneumococcal pneumonia was described early in the century (1). The loss of chlorides from the blood and their subsequent excretion in the urine during recovery was shown to be associated with retention by the body of sodium and calcium while potassium and magnesium were excreted normally or in excess.

After antipneumococcal horse serum became available for treatment of pneumonia, serum sickness developed frequently in patients during convalescence. The clinical picture of serum disease with edema and fever was shown to be accompanied by the retention of chlorides and water in the body (2). Further investigation of serum disease in human beings revealed that the clinical symptoms are associated with the appearance in the blood of precipitins against the species of animal in which the therapeutic serum was prepared (3). The appearance of precipitins precedes recovery from the disease by a short interval and is coincident with disappearance of the antigen. These studies indicated that antigen-antibody reactions were of clinical significance in the production of disease in human beings, and that the mechanism was in some way related to that of experimental anaphylaxis in animals.

During the course of studies on patients with Rocky Mountain spotted fever, it was observed that alterations in the distribution of body fluids with the development of marked clinical edema reached a maximum just before clinical recovery begins. The alterations in distribution of fluid appear to predispose to peripheral circulatory collapse. In such crises the blood chlorides, blood volume and extra-vascular thiocyanate space are altered transiently but return to normal with recovery (4). Observations in one patient who had been given hyperimmune rabbit antiserum for Rocky Mountain spotted fever showed that the alterations in the blood volume and thiocyanate space, which occurred during classic serum sickness following recovery from the initial disease, resembled strikingly those seen in the same patient during the acute phase of the rickettsial infection. These observations suggested the possibility that an immune reaction may be directly or indirectly responsible for the changes in the permeability of membranes which permit alterations in fluid distribution in rickettsial spotted fever.

The present experiments were undertaken to determine if changes in the blood volume and thiocyanate space occur in an antigen-antibody reaction induced *without infection* and to relate the physiologic alterations to the development of immunity. The anatomic changes occurring with serum sickness have been well studied, but the physiologic alterations induced by the disease are very poorly understood (5). Accordingly, rabbits were injected with human plasma and the changes in distribution of body fluids were followed serially.

## MATERIAL

Domestic rabbits of mixed breeds, weighing 2 to 4 kilograms each, were placed in individual cages and fed a stock diet, supplemented once weekly by fresh green vegetables. Water was given without restriction.

The same sample of pooled human plasma was used as the sensitizing antigen and as antigen for the precipitin tests in each individual experiment.

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[1] This study was supported (in part) by research grants from the John and Mary R. Markle Foundation, the National Heart Institute of the National Institutes of Health, Public Health Service, and the Atomic Energy Commission.

## METHODS

*Chemical techniques:* The *plasma volume* was determined by the T-1824 (Evans Blue) dye method. Syringes holding 2 ml. were calibrated to deliver 0.5 ml. of a solution containing 3 mg. of the dye per milliliter. The *thiocyanate space* was determined simultaneously. Syringes holding 10 ml. were calibrated, and 2.0 ml. of a solution containing 50 mg. of sodium thiocyanate per milliliter were injected.

The concentrations of the dye in rabbit serum were determined by the Evelyn photoelectric microcolorimeter and the thiocyanate concentrations with the Evelyn photoelectric macrocolorimeter (6). *Hematocrit* readings were made on oxalated blood placed in Wintrobe tubes, centrifuged at 3,000 revolutions per minute for 30 minutes.

The *total blood volume* was calculated from the plasma volume and the hematocrit.

*Immunologic techniques:* The humoral antibody titer was followed by ring *precipitin tests.* The human plasma was diluted 1:100 in water and used as antigen to overlay rabbit serum serially diluted in 2 × 20 mm. tubes to a maximum of 1:32. In the definitive experiment human plasma was diluted in physiologic saline, but the rabbit serum was undiluted. In the preliminary experiments dilution of human plasma (antigen) as high as 1:100,000 gave positive tests with undiluted rabbit serum (antibody) as definite as those obtained with 1:100 dilution of antigen and 1:32 dilution of antibody.

The dye was diluted as much as 1:50,000 for precipitin tests in group 1; no precipitins were encountered, so the test was not performed in later groups.

Skin tests to the dye were never positive in group 1; so they were not performed in later groups. No skin tests were done with plasma in any group.

*Experimental procedure:* In the morning before feeding the animals were weighed and strapped on their backs to wooden boards. In groups 1 and 2 blood was withdrawn without anesthesia in an oiled syringe by cardiac puncture.

Comparison of data indicated that reliable results could be obtained without deaths from hemopericardium by nicking an ear vein with a knife and collecting the blood in dry test tubes. Accordingly in group 3, 2 ml. were collected and allowed to clot; 1 ml. was then collected in another dry tube containing 6 mg. of ammonium oxalate and 4 mg. of potassium oxalate. The dye was injected into a vein in the opposite ear, the syringe changed, and the sodium thiocyanate injected through the same needle within one minute. Ten minutes following completion of the injection, 2 ml. of blood were collected from the first ear.

*Preliminary experiments:* No data were available on the reliability of the technical methods in small animals, on the effect of repeated bleedings at short intervals, or on the normal values for plasma volume and thiocyanate space in rabbits. It was not known whether rabbits might become sensitized after repeated injections of the dye and hence develop a picture analogous to serum sickness.

Accordingly, six animals (group 1) were used as a control group on the technical procedures. Determinations of the blood volume, thiocyanate space and precipitin titers, as well as a skin test, were done simultaneously every second or third day. The animals received no plasma. They were kept under observation for a minimum of 30 and a maximum of 46 days. A minimum of nine and a maximum of 16 determinations were done on each individual animal.

A second experiment was performed in order to study the variability of the physiologic changes in individual animals ill with serum sickness. It was also necessary to determine the optimum route of sensitization, the required dose of plasma and the time relationship of any observed alterations to the development of humoral immunity.

Accordingly, 11 animals (group 2) were injected with plasma. The two groups were treated in the same fashion except for the sensitizing injections of plasma. After a baseline for the individual animal had been established, the rabbits in group 2 received injections of plasma ranging from 2.2 to 5.0 ml. per kilogram of body weight. Eight animals received the sensitizing dose intravenously and three were injected subcutaneously in the intrascapular region. Three of the former animals received a second injection intravenously on the day following the initial injection.

During the clinical peak of the disease, determinations of the blood volume and thiocyanate space were done on two successive days. All animals in group 2 were followed at least 24 days following the injection of plasma, unless accidental death from hemopericardium supervened. A minimum of seven determinations were done on each animal in group 2 after the baseline was established.

In order to interpret the data critically a satisfactory statistical method of analysis had to be devised. It was necessary to determine whether groups could be compared or whether each individual animal must serve as its own control. The data from groups 1 and 2 were analyzed by several techniques and the results obtained were submitted to an impartial arbiter for criticism.[2] A satisfactory statistical method was devised and the definitive experiment designed to improve the statistical significance of the data obtained in the preliminary experiments.

*Definitive experiment:* Twelve rabbits (group 3) of more uniform size, weighing approximately 2 kilograms each, were injected with human plasma. The chemical and immunologic techniques and experimental procedure as described above were followed. At least two baseline determinations were made every second day on each animal. After the second determination, each animal was injected intravenously with a single dose of 3 ml. of plasma per kilogram of weight. Precipitin tests were performed daily on each rabbit, but no further fluid volume determinations were done until the day that a positive precipitin reaction was first obtained. The measurements were repeated on that day, on two consecutive days thereafter, and at varying intervals subsequently until the chemical values had returned approximately to the baseline.

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[2] We are deeply indebted to the Institute of Statistics of the University of North Carolina, Raleigh, N. C., for critical review of the data and for suggesting the method subsequently used.

*Statistical analysis:* The total blood volume, plasma volume, hematocrit and thiocyanate space were recorded in absolute values. The mean of two successive baseline determinations on each animal was compared with the mean of the two successive determinations made on the day the precipitins became positive and on the following day. The mean differences between the baseline values and those obtained after the precipitin test became positive were calculated. The significance of the mean difference was tested by the following formulae in which $\bar{d}$ = mean difference, $s_{\bar{d}}$ = standard error of the mean difference, S = summation of, n = number of animals:

$$t = \frac{\bar{d}}{s_{\bar{d}}} \qquad s_{\bar{d}} = \sqrt{\frac{Sd^2 - \frac{(Sd)^2}{n}}{n(n-1)}}.$$

The calculated value for t was compared with the value at n − 1 degrees of freedom as obtained from standard tables.

The data as obtained in absolute values were then calculated in terms of milliliters per kilogram of body weight on the day the measurement was made. The statistical significance was determined by the same formulae.

In view of the fact that the experiment was planned to evaluate changes in a febrile illness occurring in a growing animal the difference in the amount of food and water ingested by the animals unavoidably introduced variables which were difficult to control. Accordingly, the data were re-calculated in terms of milliliters per kilogram of *initial* body weight (the day of the first baseline determination) in order to avoid consideration of differences in food and water intake and in order to permit ready comparison with the data in group 1. The statistical significance was then determined by the same method.

It should be noted that in the definitive experiment no attempt was made statistically to compare the experimental group as a whole with the control group. Each animal served as its own control.

## RESULTS

*Control on methods:* None of the animals in group 1 showed any symptoms or signs suggesting serum sickness. No reaction was noted in any rabbit to the repeated intravenous injection of dye and no evidence of sensitization was detected by skin or precipitin tests. The fatal dose of T-1824 dye was found to be 3 mg. per kilogram of body weight given intravenously; death occurred within 15 minutes.

Moderate variations in *plasma volume, thiocyanate space* and *total blood volume* per kilogram of body weight and in the *hematocrit* were noted among the different animals. In serial determinations on a single rabbit, however, the results were much more uniform. When the data obtained near the end of the experiment were examined, a slight decrease in the thiocyanate space in relation to

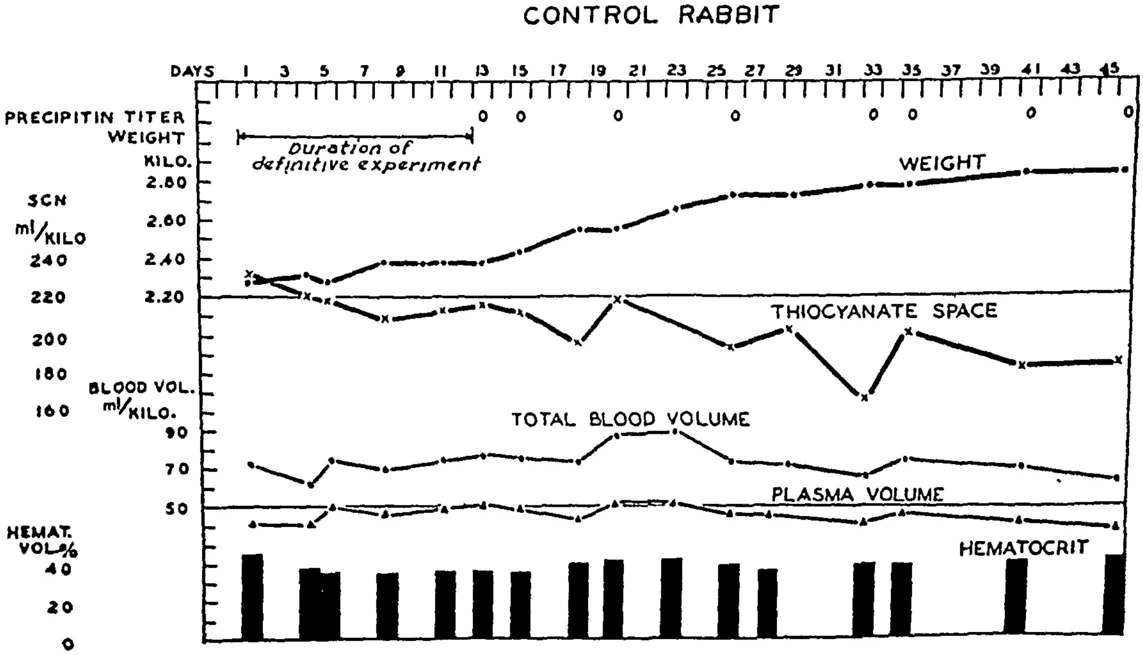

FIG. 1. FLUID SPACES IN A CONTROL RABBIT (GROUP 1) NOT INJECTED WITH PLASMA

All of the serial determinations are plotted in this graph; only four values which fall within the indicated duration of the definitive experiment are used in the calculations in Tables I and II.

TABLE I

*Control of technical methods (group 1)*

| Rabbit number | Baseline |  |  |  |  | Time of expected response |  |  |  |  |
|---|---|---|---|---|---|---|---|---|---|---|
|  | Weight | Thiocyanate space | Plasma volume | Total blood volume | Hematocrit | Weight | Thiocyanate space | Plasma volume | Total blood volume | Hematocrit |
|  | *Kilo.* | *ml./Kilo.* | *ml./Kilo.* | *ml./Kilo.* | *Vol. %* | *Kilo.* | *ml./Kilo.* | *ml./Kilo.* | *ml./Kilo.* | *Vol. %* |
| 1 | 2.30 | 219.6 | 44.2 | 68.8 | 36.0 | 2.39 | 216.1 | 49.4 | 75.5 | 34.5 |
| 2 | 3.04 | 197.8 | 47.0 | 68.8 | 31.3 | 3.04 | 197.0 | 55.8 | 87.2 | 35.7 |
| 3 | 2.53 | 210.3 | 51.3 | 73.5 | 30.0 | 2.78 | 193.8 | 49.8 | 72.8 | 31.5 |
| 4 | 3.27 | 177.5 | 43.0 | 63.5 | 33.3 | 3.24 | 162.5 | 40.0 | 62.1 | 35.5 |
| 5 | 2.76 | 207.7 | 49.7 | 77.3 | 35.8 | 2.93 | 198.1 | 47.9 | 73.3 | 34.5 |
| 6 | 2.73 | 155.3 | 42.7 | 67.0 | 36.3 | 2.92 | 162.2 | 45.0 | 62.7 | 28.0 |
| Mean for group | 2.77 | 194.7 | 46.3 | 69.8 | 33.8 | 2.88 | 188.3 | 48.0 | 72.3 | 33.3 |
| s | 3.05 | 24.00 | 3.62 | 4.90 | 2.68 | 0.29 | 21.50 | 5.27 | 9.27 | 2.99 |
| $s_x$ | 0.14 | 4.69 | 1.47 | 2.00 | 1.08 | 0.12 | 8.80 | 1.02 | 3.78 | 1.22 |

The mean of two consecutive determinations at the start of the experiment is compared with the mean of two consecutive determinations in the same animal six to eight days after the baseline was established. No injections of plasma were made.
s = Standard deviation
$s_x$ = Standard error of the mean

weight was observed; this finding would be expected as the animals increased in size from normal growth. Figure 1 illustrates the serial values in a representative control rabbit.

The mean of two consecutive baseline determinations is recorded in Table I and compared with the mean for two consecutive determinations done between six and eight days after the second determination, the time interval at which a positive precipitin test would be expected were the animals injected with plasma. The statistical analysis is summarized in Table II and indicates that, in absolute values or in milliliters per kilogram of body weight on the day of the determination, no significant difference was found. This statement is true for plasma volume, thiocyanate space, total blood volume, hematocrit, and weight.

These data have subsequently been extended in a series of 45 animals in conjunction with other studies on normal values in rabbits (7).

*General observations:* Clinical serum sickness, manifested by erythema, edema, or both at the base of the ears, generalized subcutaneous edema and hyperirritability developed in 10 of the 11 animals in group 2, and in 11 of the 12 animals in group 3. Clinical signs usually began on the fifth or sixth day following the initial injection of plasma and usually lasted two or three days. The edema was most striking in the skin over the abdomen, but was not evident unless the fur had been clipped; the skin appeared pale and felt soggy, hot, and moist. Fever (rectal temperature over 40 C.) was not a constant feature of clinical serum sickness. The clinical severity of serum sickness bore no relation to the size of the sensitizing dose. Multiple injections produced no more severe reactions than did single injections. The subcutaneous route of sensitization was as effective as the intravenous route, but variations in absorption could not be detected or controlled.

TABLE II

*Statistical significance of controls on methods (group 1)*

|  | Weight | Thiocyanate space | Plasma volume | Total blood volume | Hematocrit |
|---|---|---|---|---|---|
|  | *Kilo.* | *ml.* | *ml.* | *ml.* | *Vol. %* |
| $\bar{d}$ | +.11 | +4.33 | +10.00 | +15.33 | +.50 |
| $S_{\bar{d}}$ | .05 | 13.60 | 5.30 | 9.35 | 1.81 |
| t | 2.20 | .32 | 1.89 | 1.64 | .28 |
|  |  | *ml./Kilo.* | *ml./Kilo.* | *ml./Kilo.* |  |
| $\bar{d}$ |  | −6.42 | +1.67 | +2.45 |  |
| $S_{\bar{d}}$ |  | 3.67 | 1.90 | 3.58 |  |
| t |  | 1.75 | .88 | .68 |  |

No significant difference is noted between the baseline values and those obtained at the time of the expected response. The data are calculated in absolute values and in terms of weight on the day of the determination. The value of t was not considered significant if P was greater than .01.

*Preliminary experiments:* The alterations in plasma volume, thiocyanate space, total blood volume, and hematocrit which were observed in a representative rabbit sensitized with human plasma are illustrated in Figure 2. The changes in fluid distribution bore no direct relationship to the sensitizing dose of plasma, but paralleled the clinical severity of the disease.

In 10 of the 11 animals in group 2 increases in the *thiocyanate space* developed at some time during the period of observation. In eight animals two rises occurred, the first usually between the third and sixth days, and the second between the 11th and 20th days. The first rise usually coincided with the onset of the clinical manifestations; the second was usually marked by an increase in the subcutaneous edema. The changes in the thiocyanate space usually paralleled the weight changes, especially with the second reaction. Certain discrepancies were noted, however.

The most profound drop in *plasma volume* was noted in those animals which developed the severest clinical signs of serum sickness and the greatest and most prolonged increases in thiocyanate space. In seven animals there was no significant decrease in plasma volume, however.

In the four animals which showed a definite drop in plasma volume an appreciable decrease in the *total blood volume* also occurred. In four animals no changes in the total blood volume occurred during the experimental period.

In all the animals with severe early clinical manifestations of serum sickness a drop in *hematocrit* was associated with a rise in thiocyanate space and a decrease in plasma volume. In six animals no striking variations in the hematocrit occurred

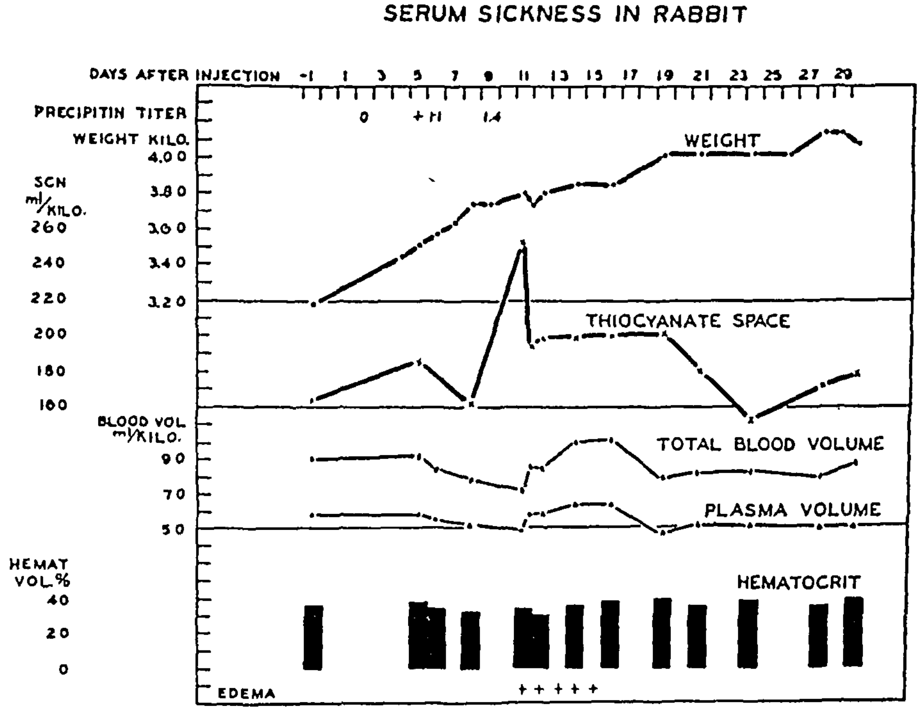

FIG. 2. FLUID SPACES IN A RABBIT (GROUP 2) GIVEN AN INTRAVENOUS INJECTION OF HUMAN PLASMA

Note the increase in thiocyanate space, which is out of proportion to the slight increase in weight, on the 11th day and the associated decrease in the plasma and total blood volume. On the following day, there was a marked decrease in the thiocyanate space, which was out of proportion to the decrease in weight. All the thiocyanate values from the 11th to the 21st day were elevated. After the 21st day, the variations in the thiocyanate space paralleled the weight changes and were not significant.

In the statistical calculations in the definitive experiment (group 3), Tables III and IV, two baseline values, two values obtained at the time precipitins appeared, and two values obtained at the termination of the experiment were used.

TABLE III

*Alterations in fluid spaces in rabbits ill with serum sickness after an injection of human plasma (group 3)*

| Rabbit number | Baseline |  |  |  |  | Response* |  |  |  |  |
|---|---|---|---|---|---|---|---|---|---|---|
|  | Weight | Thio-cyanate space | Plasma volume | Total blood volume | Hemato-crit | Weight | Thio-cyanate space | Plasma volume | Total blood volume | Hemato-crit |
|  | *Kilo.* | *ml./Kilo.* | *ml./Kilo.* | *ml./Kilo.* | *Vol. %* | *Kilo.* | *ml./Kilo.* | *ml./Kilo.* | *ml./Kilo.* | *Vol. %* |
| 18 | 2.10 | 228.5 | 46.0 | 75.5 | 39.0 | 2.10 | 322.5 | 48.0 | 74.0 | 35.0 |
| 19 | 2.14 | 235.5 | 48.5 | 80.0 | 40.0 | 2.14 | 242.5 | 47.0 | 75.5 | 37.5 |
| 20 | 1.99 | 246.5 | 48.5 | 79.5 | 39.0 | 2.14 | 282.5 | 39.5 | 61.5 | 35.7 |
| 21 | 1.87 | 245.5 | 45.0 | 75.5 | 40.5 | 1.86 | 296.0 | 39.0 | 62.5 | 37.5 |
| 22 | 1.74 | 289.5 | 48.5 | 78.5 | 37.5 | 1.96 | 273.0 | 44.0 | 68.5 | 35.2 |
| 23 | 1.87 | 266.0 | 45.5 | 74.0 | 38.5 | 2.04 | 313.5 | 40.0 | 62.0 | 36.0 |
| 24 | 1.76 | 229.5 | 50.0 | 80.0 | 37.0 | 2.05 | 281.5 | 35.0 | 54.5 | 36.0 |
| 25 | 2.10 | 212.5 | 51.0 | 84.0 | 39.5 | 2.30 | 265.0 | 39.0 | 62.0 | 36.7 |
| 26 | 2.22 | 183.0 | 54.0 | 88.5 | 39.0 | 2.24 | 225.5 | 40.5 | 62.5 | 35.7 |
| 27 | 1.76 | 215.0 | 63.0 | 108.0 | 42.0 | 1.93 | 280.0 | 47.0 | 78.0 | 40.0 |
| 28 | 1.96 | 202.5 | 52.0 | 85.5 | 39.5 | 2.07 | 307.5 | 40.5 | 63.5 | 36.0 |
| 29 | 1.85 | 220.5 | 54.0 | 92.0 | 41.0 | 1.90 | 311.0 | 48.5 | 78.0 | 38.5 |
| Mean for group | 1.95 | 231.2 | 50.5 | 83.4 | 39.4 | 2.06 | 283.4 | 42.3 | 66.9 | 36.7 |
| s | 0.17 | 28.60 | 4.95 | 9.47 | 1.38 | 0.13 | 28.00 | 4.40 | 8.44 | 1.48 |
| $s_x$ | 0.05 | 8.21 | 1.45 | 2.73 | 0.40 | 0.04 | 8.42 | 1.27 | 2.54 | 0.42 |

* The mean of two consecutive determinations at the start of the experiment is compared with the mean of two consecutive determinations in the same rabbit done on the day precipitins to human plasma appeared and on the next day.

s = Standard deviation

$s_x$ = Standard error of the mean

with the initial increase in thiocyanate space. None of the animals revealed a decrease in hematocrit in association with the second rise in thiocyanate space.

TABLE IV

*Statistical significance of alterations in fluid spaces in rabbits ill with serum sickness (group 3)*

|  | Weight | Thio-cyanate space | Plasma volume | Total blood volume | Hemato-crit |
|---|---|---|---|---|---|
|  | *Kilo.* | *ml.* | *ml.* | *ml.* | *Vol. %* |
| $\bar{d}$ | +.11 | +126.00 | −11.08 | −24.67 | −2.73 |
| $S\bar{d}$ | .03 | 21.40 | 2.81 | 4.57 | .23 |
| t | 3.70 | 5.89 | 3.94 | 5.40 | 11.87 |
|  |  | *ml./Kilo.** | *ml./Kilo.* | *ml./Kilo.* |  |
| $\bar{d}$ |  | +51.30 | −8.50 | −16.50 |  |
| $S\bar{d}$ |  | 9.85 | 1.49 | 2.60 |  |
| t |  | 5.21 | 5.70 | 6.35 |  |
|  |  | *ml./Kilo.†* | *ml./Kilo.* | *ml./Kilo.* |  |
| $\bar{d}$ |  | +74.16 | −6.25 | −12.25 |  |
| $S\bar{d}$ |  | 9.98 | 1.23 | 2.35 |  |
| t |  | 7.43 | 5.08 | 5.21 |  |

* Calculated in terms of weight on the day of determination.

† Calculated in terms of weight on the day of the first baseline.

The data all show statistically significant differences between the baseline values and those obtained at the time of the humoral antibody response. The value of t was considered significant if P was <.01.

In all 11 animals positive *precipitin tests* to human plasma developed between the third and the 12th days following the initial injection of plasma. In eight animals the thiocyanate space decreased and the clinical symptoms and signs subsided as the precipitin tests became positive. In all animals except one the precipitin tests remained positive throughout the period of observation. In this animal, which was followed longer than the others, the precipitin test was negative after the 28th day.

*Definitive experiment:* The alterations in plasma volume, thiocyanate space, total blood volume, hematocrit and body weight are summarized in Table III.

The statistical analyses are summarized in Table IV.

A statistically significant increase in *thiocyanate space* was noted in each animal at the time the humoral antibody was first detected. In all instances a return to baseline values had occurred by the 20th day after injection of the sensitizing dose. A significant increase in body *weight* occurred during the period of alteration in thiocyanate space. The body weight continued to increase from normal growth after the animal recovered from serum disease.

In contrast to the increase in thiocyanate space and weight, a statistically significant decrease in *plasma volume, total blood volume* and *hematocrit* occurred in all animals.

All of the data are statistically significant, whether the calculations are based on the comparison of *1*) absolute values; *2*) values based on milliliters per kilogram of weight on the day of the determination; or *3*) values based on milliliters per kilogram of weight on the day of the first baseline measurement.

In order to determine whether these changes were reversible, the mean of two consecutive determinations at the termination of the experiment was compared with the mean baseline values in each animal. The mean differences for the body weight, plasma volume, total blood volume, and the thiocyanate space were not significant. The decrease in the hematocrit at this time was significant. These data are not included in the tables.

In all 12 animals positive *precipitin tests* to human plasma developed between the fourth to the eighth day after injection. The distribution was as follows:

| | Onset of positive precipitin tests | | | | |
|---|---|---|---|---|---|
| Days after injection | 4 | 5 | 6 | 7 | 8 |
| Number of rabbits | 2 | 1 | 2 | 4 | 3 |

## DISCUSSION

The experiments indicate that immune reactions may be associated with alterations in the blood volume and the fluid space which is available for the diffusion of thiocyanate ion. The maximum alterations in rabbits sensitized with pooled human plasma occurred at the time circulating humoral antibodies (precipitins) appeared in the serum. This finding correlates well with the previous clinical observation that the symptoms of serum sickness begin to disappear with the appearance of precipitins (3).

The greatest change was observed in the thiocyanate space. Alterations in the plasma volume and the hematocrit were less pronounced in magnitude and were more transient, lasting only one to three days as compared with the changes in thiocyanate space which occasionally persisted into the second week. The weight of the animals tended to increase with the increase in thiocyanate space, though the changes could not always be correlated closely. Apparently the normal distribution of fluid in the body was altered as the result of an increase in permeability of membranes which was completely reversible.

*Methods:* The chemical methods were reliable since the results in the control animals (group 1) were consistently reproducible. The possibility that the dye might act as an antigen, producing serum sickness, or as a haptene, forming a new compound which might induce an auto-antibody response, was ruled out by the preliminary experiments.

The variability between individual animals in the experimental group (group 3) was controlled by determining baseline values on each rabbit and comparing these values with the response in the same animal for the statistical analysis. The effect of growth and of variations in food and water intake was apparently of little significance in this experiment, since the data were found to be statistically significant whether calculated in terms of absolute values or in relation to weight at the time of the first baseline determination or on the day of the immunologic and clinical response.

Though whole plasma is a complex of many protein fractions, each of which might induce its own slightly different immunologic response, it was felt that a mixed antigen might more closely simulate a natural infection in which multiple antigens are liberated by the infecting organism. Anatomic studies have indicated that the body responds at different times after the injection of albumin or globulin fractions derived from the same plasma (5). The two peaks in the thiocyanate space observed in the preliminary experiments may be due to this difference.

*Permeability of membranes:* The present experiment offers a logical explanation for the phenomenon of retention of salt and water; if the permeability of membranes to ions is increased, these ions could diffuse from blood into interstitial spaces and from interstitial fluid spaces into cells. The resultant hypochloremia would decrease the renal excretion of salt and water. The administration of saline would increase the edema, until restoration of the integrity of membranes corrected the alterations in the internal environment. It is not clear whether the physiologic alteration is confined solely to vascular walls, as pathologic experiments might suggest, or whether cellular membranes are also affected. Von Pirquet suggested that the edema was interstitial, but the data in the present experiments may indicate that some of the change occurs within cells (8).

*Capillary permeability:* The alterations in the vascular tree can be visualized as a series of pores in the capillary wall whose size is related to the clinical severity of the disease. In mild instances of the disease water and ions which normally traverse the membrane would leave the blood more readily. Larger molecules such as protein would be lost in more severe instances and red blood cells would pass out of the vascular tree only in the most severe reactions. Petechial hemorrhages in organs have been recognized for years as a manifestation of anaphylactic reactions.

The loss of water and ions from the vascular tree into tissues is compatible with the observations on retention of water, sodium and chloride during pneumonia and serum disease (1, 2). The permeability of the vascular tree to some colloids must have been increased because the decrease in plasma volume was concomitant with the increase in thiocyanate space. The colloids lost from the circulation to the interstitial spaces would tend to retain water extravascularly until they had been reabsorbed, presumably through the lymphatics, broken down in the tissues, or until other homeostatic mechanisms had been operative.

It is probable that the amount of protein lost from the vascular tree during serum disease is not great. The dye is known to be bound to albumin, one of the smaller protein molecules of the blood. Any great loss of albumin into interstitial spaces would reduce the amount of circulating dye and hence lead to a larger value for plasma volume by the chemical method; a loss in blood volume of any magnitude would thus be obscured. If protein was lost from the vascular tree, the defect was detected only with the first rise in thiocyanate space and was transient.

The drop in hematocrit though slight may indicate a loss of red blood cells from the vascular compartment. The drop was apparently not due to repeated bleeding since the hematocrit remained constant in group 1 in which more determinations were done in the same interval of time. No jaundice was noted clinically and the serum was never icteric, hence it seems unlikely that the red cells were hemolyzed. It is possible that the red cells were reduced in size or that they were pooled in some portion of the vascular tree which was not measured by the techniques used.

*Cellular permeability:* It is not known whether the thiocyanate ion normally diffuses into cells or not. The increase in thiocyanate space therefore could be due to *1*) interstitial edema, *2*) increase in permeability of cells to the thiocyanate ion, *3*) destruction of tissue cells with liberation of cellular water, or *4*) dehydration of cells with a shift of intracellular fluid into the interstitial spaces. The fact that some animals showed a sudden increase in thiocyanate space without an increase in body weight would favor the thesis that some redistribution of fluid had occurred whether or not additional fluid ingested in food or water was retained. The hypothesis that tissue cells were destroyed, liberating additional water and making it available for dilution of thiocyanate ion, does not seem likely. The rapidity of the increase in the thiocyanate space and its reversibility makes this explanation appear highly improbable. Such a sudden increase in thiocyanate space without an increase in body weight would have to mean that the associated tissue destruction exceeded the amount of interstitial edema. Furthermore, preliminary nitrogen balance studies in other rabbits have disclosed no marked increase in the excretion of urinary nitrogen, and presumably in cell destruction, during clinical serum sickness.

*Mechanisms:* It is not known whether the antigen in serum disease attaches solely to the vascular wall or to tissue cells outside the vascular system as well. As far as the effect on the circulation is concerned, it would be immaterial whether the loss of substances from the vascular tree is attributable to an abnormal passage of fluid between the endothelial cells, as would occur if the permeability of the intercellular cement substance were decreased, or whether the permeability of the cells themselves is abnormally increased, so that the fluids pass through the cells into the interstitial spaces.

The participation of cells in immune reactions is recognized clinically in allergic states; the deleterious antigen-antibody reaction is assumed to occur on the cell surface or perhaps within the cell. It has been postulated that histamine or some similar substance is released during the interaction of antigens with antibodies. Histamine will alter the local permeability of capillary walls. The liberation at the cell wall of some substance which would increase the permeability of the cell membrane would enhance the transmission of molecules, including the thiocyanate ion, across the surface. Such a hypothesis is of more than academic interest, since it is known that intracellular enzyme systems are affected by the extent of hydration of cells and by the concentration of ions. An increased cellular content of ions could be proved by isotopic techniques which could measure not only the diffusion of labelled thiocyanate but the relative movement of radioactive sodium, potassium, and chloride ions across cell membranes.

*Relation to infections:* The present findings indicate that the physiologic alterations which may result from antigen-antibody reactions are more profound than has been recognized in the past. The physiologic changes deserve more attention in the therapy of infectious diseases. In infections, the invading micro-organisms multiply within the host and release or secrete multiple antigens. With organisms known to locate intracellularly during some phase of the disease, as is true with the viruses and rickettsias, antigens are produced within the cell. Those antigens which could readily diffuse out would produce predominantly humoral antigen-antibody reactions; those antigens which would be retained within cells would be more likely to induce intracellular antigen-antibody reactions and hence increase the extent of the permeability of cellular walls. A most profound alteration in functions of the body could thus be produced. The possible application of antihistaminic drugs or adrenal cortical hormones to therapy of infections with an allergic element in the host response is based on such alterations in function.

## SUMMARY

1. Rabbits sensitized by the intravenous injection of pooled human plasma developed clinical edema with significant increases in weight and in the fluid space available for dilution of thiocyanate ions.
2. Significant decreases in the plasma volume, total blood volume and hematocrit were observed transiently during the most marked alteration of thiocyanate space.
3. The changes were interpreted as indicating an increase in permeability of the vascular tree, probably in the capillary wall, so that crystalloids, colloids, and red blood cells are progressively lost from the circulation as the magnitude of the defect increases with increase in the clinical severity of the disease.
4. Occasional discrepancies observed between the changes in thiocyanate space and in weight suggest that cellular permeability is probably also increased to water and ions.
5. The physiologic changes occurred at the time humoral antibodies (precipitins) appeared, suggesting that the alterations may have been due directly or indirectly to an antigen-antibody reaction.

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# INTRACELLULAR CATION EXCHANGES IN METABOLIC ALKALOSIS[1]

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(Submitted for publication October 30, 1950; accepted, February 5, 1951)

It is now well known that disturbances in the electrolyte patterns of the intracellular fluid, as well as in those of the extracellular fluid, may play a significant role in many disease states. Darrow and associates (1) have shown in experimental animals that one pattern of associated abnormalities in the two fluid phases involves intracellular potassium deficit plus sodium excess and extracellular bicarbonate excess plus deficit of chloride. Since then evidence of this relationship has been found in a variety of clinical conditions including congenital alkalosis, vomiting, the postoperative state, and syndromes of hyperadrenocortical function such as Cushing's disease and ACTH administration. This evidence has been obtained by utilization of the balance technic during replacement therapy (2–8), by study of the distribution of radioactive sodium (9), and by tissue analyses of skeletal muscle (10) and of erythrocytes (9, 11). These observations have suggested that both low potassium intake and hyperadrenocortical function are implicated in the etiology of this disturbance. In addition to these two etiological factors a third has been postulated, namely, impaired renal conservation of the potassium ion (12).

The purpose of the present study is to examine, in a group of patients with metabolic alkalosis and hypokaliemia, *1*) the direction and magnitude of intracellular electrolyte abnormalities as revealed by the balance technic, *2*) the relation of these abnormalities to the extracellular hypochloremia and elevation of bicarbonate, and *3*) the relative etiological roles of gastrointestinal fluid loss, low potassium intake, intrinsic renal dysfunction, and abnormal adrenocortical activity.

## EXPERIMENTAL MATERIAL AND METHODS[5]

Ten patients were studied by the balance technic, eight of whom were followed during fluid replacement therapy and correction of the alkalosis. The diagnoses of these ten patients and their pertinent clinical conditions are listed in Table I.

Fluids and food of known composition were administered intravenously or orally. All excreta were collected and analysed with the exception of formed stools which usually contain insignificant amounts of electrolytes when no salts have been administered by mouth. Sodium and potassium were determined in serum, urine, and nitric acid digests of stools and foods by means of a Barclay internal standard flame photometer according to the methods of Wallace, Holliday, Cushman and Elkinton (13). Chloride was determined in serum by the method of Eisenman (14), in urine by the Volhard-Harvey method (15); nitrogen was determined by micro- or macro-Kjeldahl. Total $CO_2$ content of serum and blood was measured by the method of Van Slyke and Stadie (16), and pH determinations were made with the glass electrode or colorimetrically. Creatinine in urine was determined according to the method of Brod and Sirota (17) and in serum according to the method of Bonsnes and Taussky (18). Inulin determinations in serum and urine were made according to the method of Harrison (19); spaces and clearances being determined according to the formulae, $\frac{\text{In-Out}}{\text{P}}$ and $\frac{\text{UV}}{\text{P}}$, respectively. The method for the determination of urinary 17-ketosteroids was modified from that of Holtorff and Koch (20).[6]

The derived data were calculated according to previous methods (21). The fundamental assumption involved is the equating of the extracellular fluid with the chloride space; this assumption is discussed below. The change in chloride space is calculated from the change in concentration of chloride in serum (corrected for serum water and the Donnan equilibrium), the balance of chloride, and an assumed initial or final volume for the chloride

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[1] This work was done in the Chemical and Renal Sections of the Department of Medicine and in the William Pepper Laboratory; the help of the laboratory staffs and of the clinical staff of the Hospital is gratefully acknowledged. Laboratory facilities were aided by grants from the National Heart Institute, U.S.P.H.S., and by the C. Mahlon Kline Fund of the Department of Medicine.

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[5] The laboratory assistance of Mrs. Helen Lightman and Mrs. Claire Tissari is especially acknowledged.

[6] The determinations of urinary 17-ketosteroids were done in the Endocrine Laboratory under the direction of Dr. F. Curtis Dohan.

TABLE I

*Diagnosis, pertinent history, and status of renal and adrenocortical function*

| Patient | Age | Sex | Color | Diagnosis | Pertinent history prior to study | Renal factors |  |  |  | Adrenocortical function |  |  |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
|  |  |  |  |  |  | BUN | Clearance |  |  | 17-keto-steroids | Total N balance | Remarks |
|  |  |  |  |  |  |  | Creatinine | Inulin | PAH |  |  |  |
|  | *years* |  |  |  |  | *mg. per cent* | *ml. per min.* | *ml. per min.* | *ml. per min.* | *mg. per day* |  |  |
| Group I: Low-K intake |  |  |  |  |  |  |  |  |  |  |  |  |
| W. L. | 28 | M | N | Psychoneurosis—starvation | Very little K-containing food or fluids for 4 weeks. No vomiting or diarrhea | 16–12 | — | 65 | 270 | 4.9 | Pos. | Clinical diagnosis of Addison's disease ruled out by Na conservation on 6 days low Na intake |
| F. G. | 33 | F | W | Anorexia nervosa | Semi-starvation for 5 weeks | 8–7 | 22–46* | — | — | 1.6 | Pos. |  |
| Group II: Low-K intake plus gastrointestinal fluid loss |  |  |  |  |  |  |  |  |  |  |  |  |
| A. K. | 57 | F | W | Chronic ulcerative colitis | Maintained on low K, Amigen solution i.v., for 4 weeks; extensive diarrhea | 14 | 86† | 106 | 630 | 7.3 | Neg. |  |
| N. D. | 53 | F | W | Hyperthyroidism | Vomiting, 8 weeks | 26–28 | 27–36* | — | — | 8.5 | Neg. |  |
| W. H. | 6 wks. | M | W | Congenital pyloric stenosis | Vomiting, 11 days | — | — | — | — | — | Pos. |  |
| H. H. | 67 | M | W | Cholecystitis, cholecystectomy | Postop. pyloric ileus, 12 days | 76–33 | — | — | — | 14.0 | Neg. |  |
| T. W. | 59 | M | W | Chronic hepatitis with massive liver necrosis | Gastric ileus, 10 days | 60–92 | — | — | — | — | — |  |
| A. J. | 71 | F | N | Carcinoma of colon with colostomy | Vomiting and colostomy drainage, 8 weeks | 8 | — | — | — | — | — |  |
| Group III: Adrenocortical hyperfunction |  |  |  |  |  |  |  |  |  |  |  |  |
| M. F. | 5 | M | W | Dermatomyositis | Large dosage of ACTH while on K-free fluids, 4 days | — | — | — | — | — | Neg. | 17-Ketosteroids elevated during previous course of ACTH |
| J. C. | 46 | F | W | Carcinoma of adrenal cortex with adrenogenital syndrome | Hirsutism and male habitus, 2 years; amenorrhea, 1 year; tumor in left flank | 8–11 | 55–113* | — | — | 80–85 | Pos. | No evidence of increased 11-oxysteroid activity |

* 24-hour clearance. † Acute clearance measured simultaneously with that of inulin.

TABLE II

*External exchanges of water, electrolytes and nitrogen*

| Patient | Period | | Main fluid therapy | Average daily intake | | | | | Average daily output | | | | | | | | | | Total period balance* | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | Urine | | | | | G.I. fluid† | | | | | | | | |
| | Dates | Duration | | $H_2O$ | Cl | Na | K | N | Vol. | Cl | Na | K | N | Vol. | Cl | Na | K | N | Cl | Na | K | N |
| | | *days* | | *ml.* | *m.eq.* | *m.eq.* | *m.eq.* | *gm.* | *ml.* | *m.eq.* | *m.eq.* | *m.eq.* | *gm.* | *ml.* | *m.eq.* | *m.eq.* | *m.eq.* | *gm.* | *m.eq.* | *m.eq.* | *m.eq.* | *gm.* |
| W. L. | 3/15–17 | 2 | NaCl+milk | 4,050 | 124 | 110 | 70 | 8.5 | 2,945 | 23 | 41 | 2 | 4.3 | | | | | | +200 | +136 | +135 | + 8.4 |
| | 3/17–21 | 4 | $KHPO_4$–KCl–NaCl‡ | 4,739 | 168 | 113 | 159 | 9.5 | 3,875 | 46 | 90 | 8 | 5.5 | | | | | | +482 | + 80 | +604 | +16.0 |
| | 3/21–25 | 4 | Milk | 3,713 | 62 | 46 | 85 | 10.4 | 3,455 | 61 | 107 | 44 | 8.8 | 75 | 0 | 2 | 5 | 0.4 | − 2 | −262 | +145 | + 4.7 |
| F. G. | 3/8 –11 | 3 | NaCl+Amigen | 1,938 | 163 | 200 | 1 | 7.9 | 676 | 7 | 6 | 5 | 1.7 | | | | | | +467 | +576 | − 10 | +18.4 |
| | 3/11–21 | 6 | Soft food | 2,900 | 13 | 19 | 72 | 4.5 | 887 | 12 | 39 | 4 | 1.5 | | | | | | + 5 | −128 | +407 | +17.9 |
| A. K. | 9/16–20 | 4 | $NH_4Cl$§+Amigen | 2,913 | 174 | 84 | 3 | 8.4 | 1,100 | 34 | 35 | 19 | 11.2 | 131 | 5 | 2 | 2 | 0.2 | +537 | +181 | − 73 | −12.3 |
| | 9/20–24 | 4 | KCl‖+Amigen | 3,063 | 148 | 36 | 135 | 4.5 | 575 | 46 | 25 | 41 | 2.6 | 941¶ | 42 | 36 | 32 | 6.9 | +234 | −108 | +330 | −20.1 |
| | 9/24–28 | 4 | Protenum | 1,956 | 54 | 53 | 65 | 11.5 | 554 | 28 | 20 | 23 | 3.9 | 455 | 30 | 29 | 25 | 4.1 | − 20 | + 5 | + 70 | +14.0 |
| N. D. | 7/8 –11 | 3 | $KHPO_4$–KCl–NaCl‡+milk | 4,428 | 201 | 193 | 139 | 9.3 | 2,618 | 125 | 159 | 65 | 11.9 | | | | | | +225 | + 96 | +224 | − 7.9 |
| | 7/11–13 | 2 | KCl+milk | 3,970 | 180 | 97 | 174 | 10.4 | 2,340 | 69 | 92 | 72 | 11.8 | 265 | 17 | 11 | 7 | 1.9 | +187 | − 16 | +191 | − 6.5 |
| W. H. | 8/24–27 | 3 | KCl‖+milk | 1,190 | 55 | 50 | 18 | 2.3 | 502 | 2 | 17 | 2 | 0.3 | 272 | 22 | 8 | 7 | — | + 94 | + 75 | + 25 | + 2.3 |
| | 8/27–31 | 4 | Milk—postop. | 672 | 22 | 18 | 17 | 2.4 | 145 | 1 | 7 | 2 | 0.3 | 48 | 4 | 2 | 1 | — | + 68 | + 36 | + 53 | + 7.6 |
| H. H. | 1/19–21 | 2 | $NH_4Cl$§+KCl | 4,163 | 474 | 307 | 40 | 0 | 1,150 | 7 | 77 | 55 | 13.9 | 1,305 | 193 | 194 | 12 | 3.3 | +547 | + 68 | − 54 | −34.3 |
| | 1/21–24 | 3 | NaCl+food | 2,667 | 119 | 118 | 14 | 3.2 | 1,108 | 9 | 49 | 34 | 15.5 | 383 | 19 | 20 | 5 | 0.9 | +269 | +150 | − 73 | −39.7 |
| | 1/24–28 | 4 | KCl‖+food | 3,253 | 164 | 138 | 51 | 3.0 | 753 | 11 | 56 | 29 | 9.5 | 756 | 87 | 18 | 10 | 2.1 | +261 | +247 | + 49 | −34.1 |
| T. W. | 2/7 –10 | 3 | NaCl | 3,337 | 323 | 336 | 0 | — | 404 | 1 | 5 | 11 | — | 2,500 | 186 | 70 | 51 | — | +406 | +783 | −187 | — |
| | 2/10–12 | 2 | NaCl+KCl‖ | 4,585 | 307 | 235 | 92 | — | 150 | 4 | 7 | 2 | — | 2,250 | 226 | 67 | 16 | — | +156 | +323 | +141 | — |
| A. J. | 2/21–22 | 1 | NaCl+KCl‖+milk | 3,975 | 348 | — | 128 | — | 800 | 142 | — | 23 | — | 200 | 25 | — | 2 | — | +179 | — | +103 | — |
| M. F. | 6/7 –9 | 2 | $KHPO_4$–KCl–NaCl‡ | 735 | 41 | 20 | 46 | 1.9 | 180 | 3 | 1 | 4 | 3.7 | 350†† | 3 | 1 | 4 | 3.7 | + 16 | − 60 | + 85 | − 2.8 |
| | 6/9 –13 | 4 | $KHPO_4$–KCl–NaCl‡ | 2,394 | 62 | 36 | 64 | 6.9 | 1,386 | 43 | 95 | 25 | 7.3 | | | | | | + 71 | −240 | +156 | − 1.9 |
| J. C. | 1/30–2/2 | 3 | Milk, Low K milk | 3,833 | 57 | 52 | 37 | 7.6 | 2,142 | 102 | 120 | 34 | 8.2 | | | | | | −146 | −211 | + 9 | − 1.8 |
| | 2/2 –4 | 2 | $NH_4Cl$**+Low K milk | 3,500 | 118 | 12 | 13 | 9.9 | 925 | 21 | 16 | 13 | 7.0 | | | | | | +192 | − 11 | + 1 | + 5.9 |
| | 2/4 –7‡‡ | 3 | KCl**+milk | 4,117 | 107 | 45 | 130 | 10.3 | 1,138 ± | 36 ± | 11 ± | 17 ± | 8.0 ± | | | | | | +213 ± | +102 ± | +199 ± | + 4.1 ± |

* Balances of Cl and Na corrected for small quantities lost in serum drawn for analysis.

† Includes vomitus, gastric drainage, and unformed stools.

‡ Given as an intravenous solution containing $Cl^-$ 112, $HPO_3^{--}$ 42, $Na^+$ 74, and $K^+$ 80 m.eq. per l., respectively; "$KHPO_4$" indicates a buffered solution of $K_2HPO_4$ plus $KH_2PO_4$.

§ Given intravenously as a 2 per cent solution.

‖ Given intravenously as 0.6 per cent solution.

¶ Includes urine mixed with feces.

** Given by mouth.

†† Pleural fluid.

‡‡ Urine lost on first day of this period.

Balance data are expressed per individual period rather than cumulatively.

TABLE III

*Analyses of blood and serum, and changes in the distribution of sodium and potassium between the phases of body fluid*

| Patient | Time | | Main fluid therapy | Weight | Serum | | | | | Blood pH | Extracellular volume† | Change in | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | Extracellular | | Intracellular | | | | |
| | Date | Period duration | | | $CO_2$ | Cl | Na | K | $H_2O$ | | | Na | K | Na | K* | Na | K* | K* |
| | | *days* | | *kg.* | *m.eq. per l.* | *m.eq. per l.* | *m.eq. per l.* | *m.eq. per l.* | *gm. per l.* | | *l.* | *m.eq.* | *m.eq.* | *m.eq.* | *m.eq.* | *m.eq.* | *m.eq.* | *m.eq. per kg.* |
| W. L. | 3/15 | | | 48.0 | 45.0 | 79 | 137 | 2.4 | 939 | | 5.6 | | | | | | | |
| | 3/17 | 2 | NaCl+milk | — | 40.1 | 88 | 142 | 2.5 | 943 | 7.49 | 7.2 | +250 | + 5 | −114 | +120 | ↓ | ↓ | ↓ |
| | 3/21 | 4 | KHPO₄−KCl−NaCl | 50.9 | 30.6 | 101 | 140 | 4.0 | 929 | | 10.3‖ | +440 | +23 | −360 | +543 | | | |
| | 3/25 | 4 | Milk | 49.9 | 27.9 | 104 | 141 | 5.7 | 928 | | (10.0) | − 30 | +18 | −232 | +115 | −706 | +778 | +15.6 |
| F. G. | 3/8 | | | — | 45.5 | 74 | 127 | 2.8 | 930‡ | | 15.9 | | | | | | | |
| | 3/11 | 3 | NaCl+Amigen | — | 33.8 | 98 | 137 | 2.4 | | | 16.3 | +210 | − 6 | +366 | − 48 | | | |
| | 3/21 | 6 | Soft food | 39.6 | 31.5 | 100 | 132 | 4.2 | | | (16.0) | −120 | +28 | − 8 | +336 | − 8 | +336 | + 8.5 |
| A. K. | 9/16 | | | — | 45.9 | 80 | 136 | 2.3 | 943 | | 10.0 | | | | | | | |
| | 9/20 | 4 | NH₄Cl+Amigen | — | 27.9 | 93 | 136 | 2.3 | 945 | | 13.7 | +505 | + 9 | −324 | − 52 | ↓ | ↓ | ↓ |
| | 9/24 | 4 | KCl+Amigen | — | 23.8 | 97 | 131 | 5.0 | 938 | | 15.2 | +145 | +44 | −253 | +335 | | | |
| | 9/28 | 4 | Protenum | — | 28.4 | 94 | 133 | 4.2 | 947 | | (15.6)¶ | + 55 | +43 | − 50 | + 46 | −627 | +329 | + 5.5** |
| N. D. | 7/8 | | | — | 38.6 | 93 | 147 | 2.7 | 918 | 7.43 | 6.9 | | | | | | | |
| | 7/11 | 3 | KHPO₄−KCl−NaCl+milk | — | 38.7 | 98 | 149 | 4.0 | 923 | | 8.6 | +271 | +15 | −175 | +228 | ↓ | ↓ | ↓ |
| | 7/13 | 2 | KCl+milk | 42.8 | 30.6 | 106 | 148 | 3.7 | 917 | | (9.4) | +122 | + 1 | −138 | +205 | −313 | +433 | +10.1 |
| W. H. | 8/24 | | | 3.5 | 52.8 | 62 | 131 | 3.2 | 930‡ | | 0.6 | | | | | | | |
| | 8/27 | 3 | KCl+milk | — | 40.1 | 95 | 138 | 3.3 | 930‡ | | 1.3 | + 98 | + 2 | − 22 | + 17 | ↓ | ↓ | ↓ |
| | 8/31 | 4 | Milk | 3.6 | 23.0 | 102 | 145 | 5.9 | 930‡ | | (1.8) | + 83 | + 6 | − 47 | + 29 | − 69 | + 46 | +12.7 |
| H. H. | 1/19 | | | — | 44.6 | 78 | 140 | 3.9 | | | 8.5 | | | | | | | |
| | 1/21 | 2 | NH₄Cl+KCl | — | 36.9 | 89 | 139 | 4.0 | | | 13.0 | +630 | +19 | −562 | + 4 | ↓ | ↓ | ↓ |
| | 1/24 | 3 | NaCl+food | 68.6 | 31.0 | 93 | 132 | 3.6 | 934§ | | 14.9 | +165 | + 2 | − 5 | + 21 | | | |
| | 1/28 | 4 | KCl+food | — | 30.2 | 100 | 142 | 4.8 | | | (16.2) | +320 | +14 | −173 | +117 | −740 | +142 | + 2.1 |
| T. W. | 2/7 | | | — | 25.7 | 100 | 140 | 3.9 | 935§ | | (10.0) | | | | | | | |
| | 2/10 | 3 | NaCl | — | 40.0 | 88 | 152 | 2.8 | | 7.57 | 15.5 | +980 | + 3 | −197 | −190†† | | | |
| | 2/12 | 2 | KCl+NaCl | — | 38.7 | 77 | 151 | 3.4 | | | 19.4 | +570 | +23 | −247 | +118†† | −247 | +118†† | — |
| A. J. | 2/21 | | | — | 38.3 | 89 | 146 | 1.8 | 949 | | | | | | | | | |
| | 2/22 | 1 | KCl+NaCl | — | 41.4 | 88 | 143 | 2.2 | 946 | | | — | — | — | — | — | — | — |
| M. F. | 6/7 | | | — | 40.0 | 88 | 137 | 1.6 | 934 | 7.48 | 6.2 | | | | | | | |
| | 6/9 | 2 | KHPO₄−KCl−NaCl | 63.5 | 45.0 | 82 | 140 | 3.4 | | | 6.9 | +119 | +14 | −179 | + 78 | ↓ | ↓ | ↓ |
| | 6/13 | 4 | KHPO₄−KCl−NaCl | 56.0 | 26.0 | 95 | 134 | 4.2 | 928 | | (6.5) | − 97 | + 3 | −143 | +157 | −322 | +235 | + 9.1 |
| J. C. | 1/30 | | | 61.3 | 31.0 | 105 | 142 | 2.9 | 937§ | | (15.0) | | | | | | | |
| | 2/2 | 3 | Milk, low-K milk | 58.7 | 32.9 | 102 | 144 | 2.7 | | | 14.1 | −100 | − 6 | −111 | + 20 | ↓ | ↓ | |
| | 2/4 | 2 | NH₄Cl+low-K milk | 58.7 | 29.3 | 104 | 141 | 2.7 | | | 15.5 | +160 | + 4 | −171 | − 17 | −282 | + 3 | — |
| | 2/7 | 3 | KCl+milk | 59.3 | 27.0 | 106 | 143 | 4.3 | 937§ | | — | — | — | — | — | — | — | — |

* In excess of nitrogen.
† Calculated by changes in chloride balance forward or backward from an assumed extracellular volume (designated by parentheses).
‡ Assumed.
§ Calculated from the concentration of serum protein.
‖ Inulin space measured at this time equalled 6.4 l.
¶ Inulin space measured at this time equalled 8.4 l.
** Approximated.
†† Total intracellular K, not K in excess of N.
Except where indicated by arrows, derived data are expressed per individual period rather than cumulatively.
Time as designated by date indicates end of period at which time serum analyses were made, balances for the period were determined, and exchanges calculated.

space. The inulin space was determined in two patients at the end of the period of study, but for reasons outlined in the *Discussion* were not used in the calculation of change in chloride space. From the values obtained for the volume of extracellular fluid and from the external balances of sodium and potassium, the exchanges of these two ions between the extra- and intracellular phases of the total body fluids are calculated. The results of this latter calculation are little affected by considerable variation in the volume of extracellular fluid as originally assumed or measured.

## RESULTS

The results are presented in Tables I to IV, and in Figure 1. The clinical diagnoses of the patients studied are shown in Table I.

*Intracellular cation exchanges.* In all but one (H. H.) of the ten patients potassium was retained when administered (Table II). In those patients in whom it was possible to make the calculation, the retained potassium entered the intracellular phase in excess of nitrogen, in amounts up to 15.6 m.eq. per kilogram of body weight (Table III). During treatment, in seven out of ten patients, the balance of chloride was markedly positive in relation to that of sodium (Table II). On the assumption that chloride exchanges were extracellular, it is calculated that large amounts of sodium left the intracellular phase in these patients (Table III). In some of the patients (W. L., N. D., and M. F.) the increment of potassium and the decrement of sodium were of similar magnitude; in others, more sodium was lost from the cells than potassium was gained (Table III). Only two patients were not shown to lose intracellular sodium during treatment, and in one of these, A. J., the sodium exchanges were not measured.

*Relation of intracellular cation exchanges to extracellular hypochloremia and bicarbonate elevation.* In all the cases except J. C. the elevated bicarbonate concentration in serum was associated with a lowered chloride concentration; and in all the cases during treatment the latter rose as the former fell (Table III). The high levels of bicarbonate were not associated with any marked degree of hypernatremia; during treatment in all cases except M. F. the extracellular sodium concentration remained unchanged or rose. During correction of this hypochloremic alkalosis in eight of ten cases reported the associated intracellular cation exchanges were as follows. In four cases

TABLE IV

*Relation of observed chloride exchanges to increments of chloride calculated as necessary to reduce serum $CO_2$ concentration to 31 m.eq. per l. (During early periods before response of alkalosis to therapy)*

| Patient | Period | Serum | | Chloride | | | |
|---|---|---|---|---|---|---|---|
| | | $CO_2$ | Cl | Given | Retained | Excreted in urine | Increment to lower $CO_2$* |
| | *days* | *m.eq. per l.* | *m.eq. per l.* | *m.eq.* | *m.eq.* | *m.eq.* | *m.eq.* |
| W. L. | 0 | 45.0 | 79 | | | | |
| | 2 | 40.1 | 88 | 247 | 200 | 45 | 66 |
| H. H. | 0 | 44.6 | 78 | | | | |
| | 2 | 36.9 | 89 | 948 | 547 | 13 | 77 |
| M. F. | 0 | 40.0 | 88 | | | | |
| | 2 | 45.0 | 82 | 82 | 16 | 5 | 97 |
| N. D. | 0 | 38.6 | 93 | | | | |
| | 3 | 38.7 | 98 | 603 | 225 | 374 | 67 |
| W. H. | 0 | 52.8 | 62 | | | | |
| | 3 | 40.1 | 95 | 165 | 96 | 72 | 12 |
| A. J. | 0 | 38.3 | 89 | | | | |
| | 1 | 41.4 | 88 | 348 | 179 | 142 | 124 |
| J. C. | 0 | 31.0 | 105 | | | | |
| | 3 | 32.9 | 102 | 170 | −146 | 307 | 27 |

* Calculated from the formula, $E_2 \times ([CO_2]_{S_2} - 31)$, where $E_2$ = extracellular volume at end of the period as derived from an assumed value and the balance of chloride, in liters; $[CO_2]_{S_2}$ = concentration of bicarbonate in serum at end of period in m.eq. per l.

(W. L., N. D., W. H., and M. F.) to whom potassium was administered from the start of therapy, intracellular potassium increased and intracellular sodium decreased as the bicarbonate concentration fell. In three others (A. K., H. H., J. C.) to whom $NH_4Cl$ was administered first, the bicarbonate concentration fell to normal levels and sodium was extruded from the intracellular phase in the absence of any significant uptake of potassium. In two of these three cases (A. K. and J. C.) "excess" cellular potassium was subsequently taken up when the ion was administered; in the third, H. H., potassium was not retained when given. In the eighth case, F. G., the fall in bicarbonate concentration occurred in conjunction with a rise in intracellular sodium followed by a rise in intracellular potassium. The dissociation in some of the cases of the uptake of intracellular potassium from the correction of the elevated bicarbonate concentration and its frequent association with the extrusion of intracellular sodium, as shown in three different cases, is illustrated in Figure 1.

*Etiology.* The etiological factors responsible for these electrolyte abnormalities are less readily analysed since in nine of the ten cases no observations were made during the development of the alkalosis. A rough etiological classification of the cases has been attempted in Table I. (*a*) *Low-K intake:* Two cases (Group I) gave a history of a low-K intake without any concomitant gastrointestinal fluid loss. Both of these cases showed a maximal renal response for conserving potassium at the time the studies were initiated (see below), and neither case showed any evidence of in-

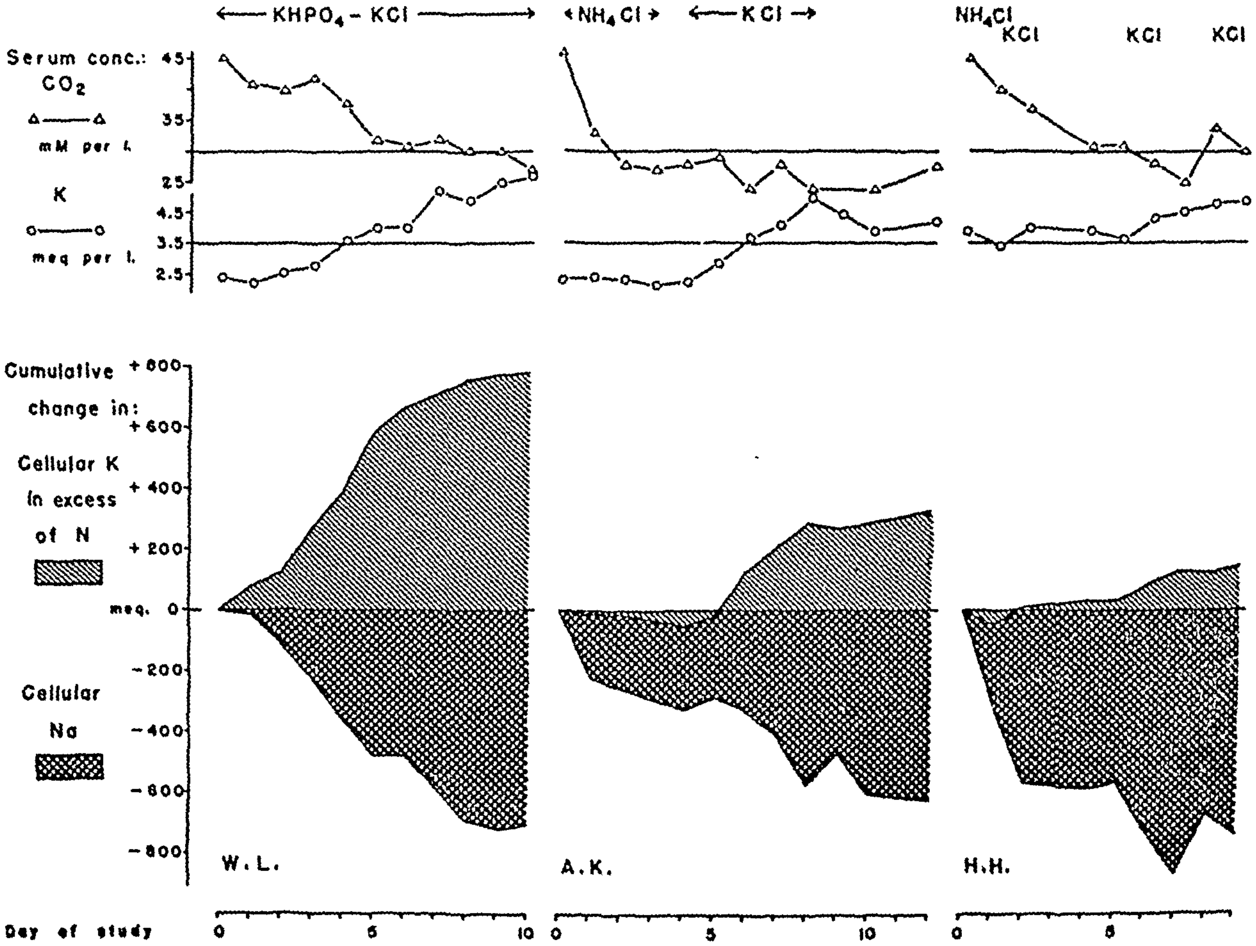

FIG. 1. RELATION OF CHANGES IN SERUM LEVELS OF CARBON DIOXIDE AND POTASSIUM TO CUMULATIVE CHANGES IN INTRACELLULAR CATIONS, DURING TREATMENT OF METABOLIC ALKALOSIS; THREE TYPES OF RESPONSE

In Patient W. L. intracellular potassium increased and sodium decreased simultaneously with the return of serum $CO_2$ and K to normal levels. In Patient A. K. during administration of $NH_4Cl$ the serum $CO_2$ level returned to normal coincidently with a loss of cellular sodium and prior to an increase in intracellular potassium. In Patient H. H. the serum $CO_2$ level returned to normal in association with a loss of intracellular sodium and without any significant subsequent uptake of cellular potassium.

creased adrenocortical activity. (*b*) *Gastrointestinal fluid loss:* Six cases (Group II) were known to have extensive losses of gastrointestinal fluid containing potassium in addition to giving a history suggestive of a low-K intake. Since, with the exception of A. K., the gastrointestinal fluid lost included gastric fluid, probably greater amounts of chloride were removed than of sodium. (*c*) *Adrenocortical hyperfunction:* In Group III are listed two cases in which this factor apparently played a major etiological role. In M. F. the administration of large doses of ACTH, in conjunction with a low intake of potassium, immediately preceded the onset of prostration associated with a severe alkalosis and hypokaliemia. This patient had been shown during a previous course of ACTH to have an adrenocortical response as evidenced by a fall in circulating eosinophils, a rise in 17-ketosteroid excretion, and retention of sodium (22). In the other case, J. C., who had a typical adrenogenital syndrome due to carcinoma of the adrenal cortex, hyperfunction of the gland was the only factor that could be adduced to account for the mild alkalosis and hypokaliemia found. In none of the other cases was there unequivocal evidence of adrenocortical hyperfunction occurring as an etiological factor although thorough and systematic studies of this aspect of the problem were not undertaken. In five of the other eight cases the excretion rate of 17-ketosteroids was determined and was found to be normal or low (Table I). A negative nitrogen balance which might indicate adrenogenital hyperfunction was found in three of these cases during treatment. Of these A. K. had an extensive loss of nitrogen from the bowel, N. D. was thyrotoxic, and H. H. was in the immediate postoperative period. It is therefore difficult to use this observation as evidence of primary adrenocortical hyperfunction. (*d*) *Renal dysfunction:* kidney function was not systematically studied in this series of cases. Certain observations in some of the cases, however, are worthy of note (Table I). Five cases did not have azotemia; two of these, W. L. and F. G., were patients who apparently developed the alkalosis and potassium deficiency as the result of a low-K intake alone. During the first few days of study at the height of the alkalosis both of these patients excreted such extremely small amounts of potassium that the U/P ratio was less than 1.0. Conservation of potassium, therefore, was maximal at that time, despite the evidence from clearances of creatinine and inulin that glomerular filtration was impaired.

## DISCUSSION

The observations made in this study confirm the hypothesis that extensive disturbances of intracellular electrolyte exchanges usually occur in states of metabolic alkalosis. Intracellular deficit of potassium, as measured by uptake of the ion during treatment, was certainly present in most of the cases. In Patient W. L. the deficit measured in this way reached a maximum of 15.6 m.eq. per kilogram of body weight, and is of the same magnitude as the largest deficits reported in infant diarrhea (23) or diabetic acidosis (24). If it is assumed that all of this potassium came from skeletal muscle and that this tissue constitutes 40 per cent of the body weight, the deficiency would be equivalent to 39 m. eq. per kilogram of wet muscle and would approximate 40 to 50 per cent of the potassium in the intracellular phase. Such a deficit is quite comparable to those produced experimentally in rats by Darrow (1) and exceeds those found in muscle biopsies taken from clinical cases by Mudge and Vislocky (10).

The demonstration of an excess of sodium in the intracellular phase is less certain. As calculated on the basis of the assumption that the chloride exchanges were entirely extracellular, the intracellular sodium exchange during treatment was negative in all but one of the nine cases in which it was measured. In four of the cases the size of the decrement of cellular sodium was sufficiently close to that of the increment of potassium to suggest that the former cation was being substituted for by the latter. In the other four cases the loss of cellular sodium so far exceeded the uptake of potassium as to obviate any such relationship. The validity of the assumption, however, on which these exchanges of intracellular sodium are calculated, must be questioned. Certainly in most of the cases during treatment the external balance of chloride was much greater in the positive direction than that of sodium. These balances are observed, not derived, data, and would not be invalidated by any unmeasured loss of salt in sweat in which chloride and sodium are present in approximately equal concentrations. The differential secretion of Cl into gastric fluid which was not lost from the body and therefore not included in the measured balance, conceivably might be another source of error. The magnitude of the discrepancies between Cl and Na renders this explanation improbable; it is unlikely that, during the course of study in some of the patients, 5 or 6 l. of unreabsorbed gastric juice accumulated undetected. The difference between the external balances of these two ions would appear to require some other explanation. An alternative to the interpretation that these disparate balances represented loss of sodium from the cells is that chloride entered the intracellular phase. Physiological evidence for such a phenomenon is not lacking. Conway's (25) theory calls for a small amount of intracellular chloride to balance the extracellular potassium, and Dean (26), Wilde (27), and Burch and co-workers (28) have presented evidence that under certain circumstances chloride may enter the cells of tissues other than gastric mucosa, testes, and ovaries where chloride is a normal intracellular constituent. Evidence in support of the entry of chloride into cells was found in three of the cases reported here, W. H., W. L., and A. K. In the infant, Patient W. H., during therapy the chloride space expanded to a volume equivalent to approximately 50 per cent of the body weight which is unexpectedly large for a non-edematous subject even in childhood. A similar finding has been described during treatment of vomiting infants by Danowski and his associates (4), who were unable to attribute it to error in measurement of the chloride balance. In Patients W. L. and A. K. an inulin volume of distribution was determined at the end of replacement therapy. When this volume of distribution was taken as the final volume of extracellular fluid and, from it, and from the chloride balance, the initial volume of extracellular fluid was calculated, the latter was found to be less than 1 l. Since such a value is absurd, the conclusion is that either the measurement of the volume of distribution of inulin was in error, or that chloride entered cells. A definitive answer to this problem requires the accurate measurement of the extracellular fluid volume by some independent means, such as inulin, both before and after treatment, so that the intracellular transfer of chloride, if any, can be measured directly. Unfortunately, an opportunity to do this in the present study was not available. The possibility remains, therefore, that chloride entered the intracellular phase during treatment in these cases. But again because of the magnitude of the transfers involved, it seems most unlikely that this did occur to such an extent as to preclude the extrusion of sodium.

Certain conclusions can be drawn in regard to the relationship of these intracellular electrolyte abnormalities to the extracellular hypochloremia and alkalosis. The production of intracellular potassium deficiency and of probable sodium excess by prolonged absence of potassium intake, appeared to result in hypochloremia and elevation of the extracellular bicarbonate concentration. This suggests that under conditions of biological equilibrium or the steady state of adequate renal adjustment, as predicted by Darrow (1), these intracellular disturbances are associated with a diminished tubular reabsorption of chloride despite the presence of hypochloremia. Such a concept receives further support from an analysis of the renal response to chloride administration in some of these cases, as presented in Table IV. In three of the cases tabulated, the extracellular fluid volume was expanding to such an extent that the administered chloride was insufficient to raise the extracellular concentration even if the kidneys had been able to conserve it, *i.e.*, the kidneys excreted less chloride then the increment calculated as necessary to displace the bicarbonate to normal levels. In other words, in these cases, the kidneys had no chance to show conditioning by another factor and the data cannot be used to imply that the circumstances required a maintenance of hypochloremia and alkalosis in the presence of chloride administration. In the other four cases of alkalosis, three of whom had hypochloremia (N. D., W. H., A. J., and J. C.), during the initial days of study chloride was excreted by the kidney in amounts sufficient to have displaced the high bicarbonate had the chloride been conserved. Thus the failure to adjust the abnormal concentrations in the latter cases was not due to unavailability of chloride when neutral salts, NaCl and KCl, were given. Pitts, Ayer and Schiess (29) have found that, during the acute administration of sodium bicarbonate, as the tubular reabsorption of $HCO_3$ rises to a maximal rate, the reabsorption of chloride is correspondingly depressed; and as a result the total cation concentration in the extracellular fluid is undisturbed. The cases reported here are hardly comparable to these experimental subjects of Pitts since ours represent prolonged states of equilibrium maintained in the presence of conditions other than acute loading of excess sodium bicarbonate. Furthermore, if the results of the acute i.v. administration of a single dose of sodium bicarbonate to a normal subject are observed over a period of many hours the initial rise in the rate of renal excretion of chloride is followed by a fall (30); as the elimination of the excess bicarbonate continues the kidneys again conserve chloride and the extracellular concentrations return to normal. In the last four cases shown in Table IV, therefore, at a time when chloride was available some other factors must have been conditioning the tubular reabsorption of this ion.

However, when chloride ion was administered rapidly and in large quantities as an acidifying salt, $NH_4Cl$, chloride was retained in sufficient amounts to raise its extracellular concentration to normal as the extracellular bicarbonate concentration was lowered. Simultaneously, the extracellular volume or chloride space expanded and sodium moved into it from the intracellular space. This occurred without any restoration of potassium to the cells. There are at least two interpretations of these transfers. One is that an excess of intracellular sodium may have made this adjustment possible. Another is that the kidney was unable to excrete at least part of the suddenly imposed load of extra anion with ammonia because of renal damage or because of the time lag inherent in the mechanism of ammonia formation. Under these circumstances, the transfer of cellular sodium (the only sodium available) was the only way to mitigate an otherwise abrupt change in acid-base equilibrium due to displacement of bicarbonate as $CO_2$ through the lungs. Such a sodium transfer requires some accounting in regard to the intracellular anion pattern; this problem is discussed below. But whichever of the above interpretations is accepted, of the response to $NH_4Cl$, it would appear that if there is an obligatory relationship between the extracellular concentrations of chloride and bicarbonate and intracellular cation compositions, the relationship pertains more directly to intracellular sodium than to potassium.

According to the Darrow hypothesis, the direct withdrawal of chloride in excess of sodium by way of gastric fluid, resulting in a metabolic alkalosis, should produce changes in intracellular cation composition even in the presence of an adequate intake of potassium. Unfortunately our data are incomplete on this point. Only in Patient T. W. were balance studies obtained under such conditions. Intracellular potassium was diminished because of the loss in the gastric washings, but intracellular sodium was not increased. This may be accounted for by the presence of moderately severe renal insufficiency which certainly interfered with any renal readjustments that might have been made. Prior to study, Patient H. H. had lost a large amount of gastric fluid by lavage. Studies during his replacement therapy showed a very large decrement of cellular sodium as his bicarbonate concentration fell, without much retention of potassium. Thus under some circumstances extracellular chloride deficit and alkalosis probably can be produced without necessarily affecting either the sodium or potassium content of the intracellular phase. This is in agreement with the experience of Evans and co-workers who found in both surgical patients and in dogs that alkalosis could occur without cellular potassium depletion and could be corrected without the administration of this ion (31–33).

Darrow has suggested that the primary factor in extracellular fluid which conditions the intracellular electrolyte pattern is the concentration of hydrogen ion rather than that of bicarbonate or chloride. In support of this thesis he has cited his experiments (34) in which rats on exposure to low atmospheric pressures developed sodium excess and a questionable potassium deficit in the cellular phase of skeletal muscle. Presumably an alkalosis developed due to primary $CO_2$ deficit with a *lowered* $CO_2$ content of extracellular fluid, although no such measurements were made. We have studied patients representing the converse of this condition, namely, those with respiratory acidosis due to the primary retention of $CO_2$ as a result of severe pulmonary disease. This condition leads to a secondary fall in chloride and rise in bicarbonate concentration as a response to the rise in level of carbonic acid. In a small series of such patients with marked elevations of serum bicarbonate concentration, but with *low blood pH,* the serum concentration of potassium was found to be uniformly within the normal range (35). This finding suggests, but does not prove, that no severe intracellular depletion of potassium coexisted with the high bicarbonate concentration found in respiratory acidosis. These studies are not adequate controls to those reported in this paper since the uptake of potassium administered to such patients was not tested. But they do suggest that intracellular electrolyte composition is conditioned not by concentrations of extracellular bicarbonate but by concentrations of hydrogen ion or carbonic acid. The latter is suggested by data of Wallace and Hastings (36) who found by direct analysis of muscle that the bicarbonate content of intracellular fluid is very little affected by the bicarbonate of the extracellular phase (the cell is impermeable to $HCO_3^-$), the calculated intracellular pH being altered according to changes in $CO_2$ pressure alone.

Extensive differential net transfers of cations versus anions between the two phases of body fluids require consideration of their effect on the ionic electrical neutrality and osmotic pressure of the respective phases. Knowledge of the ways in which electrical neutrality of intracellular fluid is maintained under these circumstances awaits at least the complete description of net exchanges across the cell boundary of the cations, calcium and magnesium, in addition to sodium and potassium, and of the anion, phosphate, as well as chloride. Some evidence on this score has been supplied by Gardner, MacLachlan and Berman (37) who found in the skeletal muscle of potassium deficient alkalotic rats, that the intracellular bicarbonate was decreased and phosphate increased, and that calcium and magnesium, as well as sodium entered the cellular phase. However, in addition to external transfers, metabolic processes and variations in cellular pH must affect the degree of dissociation of the organic phosphate and proteinate buffer system within the cell. Alterations in cellular osmolarity, so produced, apparently share in conditioning movements of water (38). Therefore, any complete accounting of the maintenance of electrical neutrality and of osmotic equilibrium is impossible with the data at hand.

From the study of these cases it appears to the authors that a variety of disturbances in intracellular cation pattern may exist in the presence of metabolic alkalosis, produced in a variety of ways. These disturbances may consist of (*a*) a decrease in potassium and an increase in sodium, (*b*) an increase in sodium without change in potassium, and (*c*) a decrease in potassium without an increase in sodium. The first of these, *a*, is most common and is usually present when there has been over-activity of the adrenal cortex or when the intake of potassium has been very low for a prolonged period of time. Under the latter circumstance the kidneys continue to excrete potassium for a long enough period to produce a significant deficiency before renal conservation takes place. Likewise, prolonged loss of gastric fluid, usually associated with inadequate potassium intake, may become associated with a decrease in potassium and an increase in intracellular sodium. On the other hand abrupt and drastic disturbance of extracellular fluid composition, such as may occur with excessive vomiting or postoperative gastric lavage, may be associated with no intracellular changes or with other patterns of change such as *b* or *c* listed above. In such conditions are more likely to be found the renal changes described by Burnett, Burrows and Commons (12) leading to potassium wasting, and in such conditions is less likely to be found the steady state of biological equilibrium (Darrow's prerequisite to intracellular pattern, *a*). It would be hazardous, therefore, to predict the precise intracellular cation disturbances in any case of metabolic alkalosis without due consideration of the physiological and clinical circumstances of its origin.

## SUMMARY AND CONCLUSIONS

By use of the balance technic the intracellular cation exchanges have been studied in ten cases of metabolic alkalosis during treatment.

In all but one of the cases potassium was retained in the intracellular phase. In seven of the ten cases the chloride balance was much more positive than that of sodium. On the assumption that chloride is extracellular, large amounts of sodium were calculated to have left the intracellular phase. In some patients the decrement of sodium was roughly equivalent to, and in others greatly exceeded, the increment of potassium.

The extracellular bicarbonate concentration fell to normal levels concurrently with the increase in potassium and decrease in sodium in the intracellular phase, in four cases. In three others when $NH_4Cl$ was given, the bicarbonate concentration fell and sodium was extruded from the cells prior to any significant uptake of potassium by the latter.

In one of these cases no potassium was retained, and in one sodium entered cells as the alkalosis was corrected.

A variety of etiological factors appeared to be involved in this group of cases as follows: low potassium intake alone, two; low potassium intake plus gastrointestinal fluid loss, six; adrenocortical hyperfunction (ACTH therapy, carcinoma), two. No evidence of abnormal renal wasting of potassium was found and in two cases an extreme degree of conservation of the ion with U/P ratios of less than 1.0 was observed.

It is concluded from these data that although decrease in potassium and increase in sodium is a common intracellular cation disturbance in metabolic alkalosis, other patterns of disturbance may occur, especially when the onset has been abrupt and renal adjustment has not taken place. Where there is an equilibrium state between extracellular bicarbonate concentration and intracellular cation transfers, the former is more directly linked to those of sodium than to those of potassium.

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# INTRACELLULAR CATION EXCHANGES IN ACIDOSIS DUE TO RENAL INSUFFICIENCY. EFFECTS OF ALKALI THERAPY[1]

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(Submitted for publication October 30, 1950; accepted, February 5, 1951)

Disturbances in the exchanges of certain intracellular electrolytes which occur in patients with metabolic alkalosis, have been described in the previous paper (1). Clues to the direction and magnitude of these disturbances were obtained by calculating from balance measurements the alterations in body fluids produced by replacement therapy. In the present communication the results are presented of a similar study in patients with metabolic acidosis due to renal insufficiency.

Since this study involved the administration of hypertonic solutions of sodium bicarbonate or sodium lactate, it seemed advisable to reassess all of the factors, including those of intracellular transfers, which determine the quantitative effect of such replacement therapy on the total fluid and acid-base equilibrium of these patients. Previous studies have been made of the effects of alkalinizing sodium solutions. Palmer and Van Slyke (2) and later Hartmann and Senn (3) found that the rise in serum bicarbonate was such that the dose of alkali was apparently distributed through a volume equal to two-thirds of the body weight, a fraction which they believed represented the total body water. Since both bicarbonate ions (4, 5) and the major portion of sodium ions are not freely diffusible across the cell membrane, some explanation must be sought for the discrepancy between the value stated above and the now commonly accepted magnitude of extracellular fluid of 15 to 20 per cent of the body weight (6).

One of the factors which might explain this difference in the data of Palmer and Van Slyke and Hartmann and Senn was the fact that the dose of administered sodium salt was not corrected for that portion which was unabsorbed or excreted. Another factor was the failure to allow for the change in protein anion concentration in red cells and plasma. Since the amount of cation which is buffered by protein changes as pH is altered, Singer and Hastings (7) have indicated that the effect of administered acid or alkali is more directly measured by the change in "buffer base," which is equivalent to the change in bicarbonate plus protein anion, than by the change in bicarbonate alone. Such calculation applied to the data of Shock and Hastings (8) gives an apparent volume of distribution of orally administered sodium bicarbonate of about 45 per cent of the body weight, instead of the larger percentage obtained by Palmer and Van Slyke.

Since it seems unlikely that these large volumes of distribution of administered sodium bicarbonate or lactate can be explained by excretion or failure of absorption of sodium, intracellular transfers would appear to be involved. Darrow and coworkers (9) observed that in the muscles of rats rendered acidotic experimentally, the intracellular sodium was depleted. On the basis of this finding he has suggested (10) that uptake of sodium by cells is the explanation of the large doses of alkali required to combat clinical acidosis. Our study confirms this hypothesis and reveals that even when the errors of absorption and excretion are eliminated, and when the buffer base is measured, the quantitative results of alkali therapy in this type of acidosis can only be explained by extensive transfers of cations across the cell boundary.

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[1] Laboratory facilities were aided by grants from the National Heart Institute, U.S.P.H.S., the C. Mahlon Kline Fund of the Department of Medicine, and the Committee on the Advancement of Research of the University of Pennsylvania. The help of the laboratory staffs and of the clinical staff of the Hospital is gratefully acknowledged.

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## EXPERIMENTAL MATERIAL AND METHODS[4]

Eight adult patients with metabolic acidosis due to renal insufficiency were studied. The diagnoses are listed in Table I. Four of these patients were in the polyuric phase of uremia and four were oliguric or anuric. The studies extended from two to ten days over the periods during which sodium bicarbonate was being administered by vein or by mouth. Balances were measured and electrolyte and water exchanges were calculated as in the previous paper (1).

In four of the patients during early periods of therapy detailed studies were made of changes in the acid-base equilibrium. This was done by the technic described by Shock and Hastings (11) which involves the micro-determination of pH and total $CO_2$ content of cutaneous blood. From these values and that of the hematocrit the tension of carbon dioxide ($pCO_2$) and the concentration of buffer base ([BB]) were calculated according to the nomogram of Singer and Hastings (7). These changes were then correlated with the data for the corresponding balance periods.

Since in most of the cases large changes occurred in the urea nitrogen concentration of body fluids, some difficulty was involved in the calculation of transfers of intracellular potassium which were "in excess of nitrogen" or unrelated to catabolism and anabolism of cells. To make this calculation the external nitrogen balance was corrected by a value obtained by multiplying the change in blood urea nitrogen concentration by an assumed value for total body water. Obviously, where the change in blood urea nitrogen concentration is large and the external nitrogen balance is small, the derived value for "excess potassium" can be but a rough approximation. Such a calculation is useful, however, in indicating at least the direction and type of exchanges involved.

## RESULTS

The results are presented in Tables I to III, and in Figures 1 and 2.

*Sodium exchanges and acid-base equilibrium.* During the administration of sodium bicarbonate, all of the patients were in a strongly positive sodium balance (Table I). The concentration of

TABLE I

*External exchanges of water, electrolytes, and nitrogen*

| Patient | Age and sex | Diagnosis | Period | Average daily | | | | | | | | | | Total period balance* | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Intake | | | | | Urinary output | | | | | | | | |
| | | | | $H_2O$ | Cl | Na | K | N | Vol. | Cl | Na | K | N | Cl | Na | K | N |
| | | | day of study | *ml.* | *m.eq.* | *m.eq.* | *m.eq.* | *gm.* | *ml.* | *m.eq.* | *m.eq.* | *m.eq.* | *gm.* | *m.eq.* | *m.eq.* | *m.eq.* | *gm.* |
| Group I: Polyuric patients | | | | | | | | | | | | | | | | | |
| E. G. | 59 F | Chr. pyelonephritis | 0–4 | 2,870 | 70 | 221¶ | 82 | 10.7 | 741 | 28 | 40 | 14 | 2.5 | +165 | +725 | +274 | +32.8 |
| C. P.† | 19 M | Chr. glomerulonephr. | 0–1 | 4,100 | 57 | 226 | 135 | 10.3 | 3,775 | 130 | 145 | 72 | 17.5 | − 80 | + 75 | + 55 | − 7.5 |
| | | | 1–2 | 3,200 | 60 | 226 | 135 | 10.7 | 2,285 | 69 | 82 | 55 | 9.1 | − 10 | +142 | + 80 | + 1.6 |
| H. H. | 70 F | Lower nephr. nephrosis | 0–7 | 3,510 | 55 | 124 | 75 | 9.3 | 2,480 | 70 | 96 | 14 | 10.1 | −111 | +185 | +429 | − 6.0 |
| A. T. | 38 F | Tubular insufficiency | 0–3 | 3,023 | 0 | 120 | 64 | 0 | 1,362 | 45 | 29 | 16 | 4.2 | −141 | +268 | +143 | −12.6 |
| | | | 3–10 | 3,747 | 99 | 108 | 117 | 4.3 | 1,802 | 88 | 93 | 42 | 8.7 | + 67 | + 86 | +536 | −30.2 |
| Group II: Oliguric and anuric patients | | | | | | | | | | | | | | | | | |
| G. D.‡ | 18 F | Dissem. lupus | 0–2 | 1,170 | 23 | 300 | 1 | — | 140 | 5 | 1 | 7 | — | − 2 | +483 | − 20 | — |
| J. R. | 36 M | Nephrosclerosis | 0–1 | 1,120 | 36 | 251 | 2 | 0 | 140 | 2 | 1 | 5 | 0.4 | + 18 | +244 | − 6 | − 0.5 |
| | | | 1–5 | 1,286 | 67 | 202 | 10 | 0.2 | 334 | 5 | 5 | 15 | 1.9 | +237 | +777 | − 24 | − 7.2 |
| M. B.§ | 65 M | Lower nephr. nephrosis | 0–6 | 1,800 | 27 | 111 | 0 | 0.5 | 105 | 7 | 12 | 2 | 0.8 | −220 | +359 | − 41 | − 9.3 |
| I. C.‖ | 40 F | Lower nephr. nephrosis | 0–8 | 1,505 | 24 | 118 | 0 | 0 | 5 | — | — | — | — | − 42 | +752 | −135 | − 9.1 |

* Balances corrected for small quantities of Cl and Na lost in serum drawn for analysis, and for electrolytes lost in gastrointestinal and pleural fluids where indicated.

† 200 ml. of vomitus included in balance.

‡ 600 ml. of pleural fluid included in balance.

§ Gastric drainage removed during last three days included in balance.

‖ Upper gastrointestinal drainage and electrolytes removed by resin enemata included in balance.

¶ Given by mouth.

Balance data are expressed per individual period rather than cumulatively.

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[4] The laboratory assistance of Mrs. Helen Lightman and Mrs. Claire Tissari is especially acknowledged.

TABLE II

*Analyses of blood and serum, and changes in the distribution of sodium and potassium between the phases of body fluid*

| Patient | Time | Weight | Serum | | | | | Blood urea N | Extracellular volume† | Change in | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | Sodium | | Potassium | |
| | | | $CO_2$ | Cl | Na | K | $H_2O$* | | | Extracell. | Intracell. | Extracell. | Intracell.‡ |
| | day of study | *kg.* | *mM. per l.* | *m.eq. per l.* | *m.eq. per l.* | *m.eq. per l.* | *gm. per l.* | *mg. per cent* | *l.* | *m.eq.* | *m.eq.* | *m.eq.* | *m.eq.* |
| Group I: Polyuric patients | | | | | | | | | | | | | |
| E. G. | 0 | 48.7 | 13.5 | 101 | 129 | 4.6 | 931 | 107 | ( 8.0) | | | | |
| | 4 | 50.2 | 23.0 | 91 | 139 | 3.6 | | 88 | 10.4 | +420 | +305 | ± 0 | +178 |
| C. P. | 0 | 66.0 | 15.8 | 97 | 132 | 5.0 | 939 | 132 | (10.0) | | | | |
| | 1 | 66.0 | 21.2 | 92 | 136 | 5.1 | | 144 | 9.8 | + 15 | + 60 | ± 0 | + 86 |
| | 2 | 66.0 | 26.5 | 95 | 141 | 5.0 | | 144 | 9.4 | − 10 | +152 | − 3 | + 79 |
| | | | | | | | | | | + 5 | +212 | − 3 | +165 |
| H. H. | 0 | — | 11.7 | 99 | 126 | 2.5 | 942 | 112 | (15.0) | | | | |
| | 7 | — | 21.6 | 103 | 139 | 2.5 | | 104 | 13.5 | − 15 | +200 | − 4 | +428 |
| A. T. | 0 | — | 3.8 | 120 | 136 | 2.6 | 936 | 15 | 7.9 | | | | |
| | 3 | — | 13.1 | 106 | 134 | 2.6 | 937 | 39 | 7.8 | − 30 | +298 | − 1 | +192 |
| | 10 | 43.6 | 25.6 | 99 | 134 | 4.1 | 934 | 20 | ( 9.0) | +164 | − 78 | +17 | +568 |
| | | | | | | | | | | +134 | +220 | +16 | +760 |
| Group II: Anuric patients | | | | | | | | | | | | | |
| G. D. | 0 | 70.0 | 10.8 | 88 | 116 | 7.1 | 957 | 152 | (15.0) | | | | |
| | 2 | — | 20.7 | 94 | 128 | 7.1 | | 152 | 14.1 | + 65 | +418 | − 7 | − 13§ |
| J. R. | 0 | — | 16.7 | 76 | 112 | 4.9 | 944 | 98 | (16.0) | | | | |
| | 1 | — | 18.9 | 75 | 117 | 5.3 | 942 | 116 | 16.4 | +125 | +119 | + 9 | + 6 |
| | 5 | — | 27.0 | 75 | 131 | 6.1 | 940 | 148 | 19.2 | +595 | +182 | +30 | − 1 |
| | | | | | | | | | | +720 | +301 | +39 | + 5 |
| M. B. | 0 | 82.0 | 19.8 | 84 | 131 | 4.9 | 928 | 83 | (17.0) | | | | |
| | 6 | — | 28.0 | 69 | 139 | 7.1 | 921 | 138 | 17.4 | +210 | +149 | +41 | + 6 |
| I. C. | 0 | — | 17.6 | 93 | 135 | 5.3 | 931 | 116 | (15.0) | | | | |
| | 8 | — | 21.6 | 76 | 143 | 5.4 | | 224 | 17.8 | +530 | +222 | +16 | − 4 |

* Calculated from the concentration of serum protein.

† Calculated by changes in chloride balance forward or backward from an assumed extracellular volume (designated by parentheses).

‡ In excess of nitrogen.

§ Total intracellular K, not K in excess of N.

Time as designated by day indicates end of period at which time serum analyses were made, balances for the period determined, and exchanges calculated.

serum sodium rose in all (Table II). In all of the patients sodium entered the intracellular or "non-chloride" phase (Table II). This increment of intracellular sodium varied from 29 to 108 per cent of the amount of sodium retained, and in four of the eight patients was more than 50 per cent. In three of the eight patients little or none of the sodium was retained in the extracellular phase; consequently, in these patients, the increase in *concentration* of extracellular cation which was available for the bicarbonate and protein anions was the result primarily of decreases in extracellular water and chloride rather than of an increase in the *absolute amount* of extracellular sodium. Thus, whether or not there was an increase in the absolute amount of extracellular sodium, all of the and of other ions. As pointed out in the preceding paper (1), any assessment of the relation of these cation transfers to maintenance of ionic electrical neutrality and to osmotic equilibrium within the cells, requires a great deal more data than is available at present. Precise knowledge of these disturbances will only come when these other factors have been more adequately studied and their relationship defined to the metabolic processes which maintain the differential distribution of electrolytes across the cell boundary.

## SUMMARY AND CONCLUSIONS

The exchanges of intracellular sodium and potassium were calculated by the balance technic in eight patients with acidosis due to severe renal insufficiency, during the administration of sodium bicarbonate. On four of the patients acid-base data were obtained.

Sodium entered the intracellular phase in all of the patients in amounts ranging from 29 to 108 per cent of the sodium retained.

Potassium entered the intracellular phase in the four polyuric patients to whom it was given. In the anuric patients to whom no potassium was given because of an elevated extracellular concentration of the ion, no significant exchange occurred of intracellular potassium in excess of nitrogen.

The metabolic acidoses as measured by the buffer base or bicarbonate were corrected in whole or in part in all of the patients.

It is concluded (*1*) that measurements of the whole blood buffer base give a better index than the $CO_2$ content of changes in this type of acid-base disturbance, (*2*) that shift of sodium into cells, as well as excretion of sodium and balance of chloride, determine the effect of administered alkali on the acidosis, and (*3*) that because of the unpredictability of these factors the therapeutic dosage can only be roughly estimated ahead of time.

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# THE LIPIDS OF SERUM IN PREGNANCY[1]

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(Submitted for publication December 9, 1950; accepted, February 5, 1951)

It has been repeatedly demonstrated that the concentration of cholesterol in the serum of women increases during pregnancy (1–8). Fatty acids (1, 6, 7) and phospholipids (4, 6, 7) also rise somewhat. There is not such general agreement concerning the time relations of the elevations nor the relative changes of the various lipid components. Bloor and Knudson (9) claimed that the increment of cholesterol is composed chiefly of esters; Gardner and Gainsborough (5) found that it consisted entirely of free cholesterol; while, according to Boyd (7), the partition of cholesterol is unaltered. These disagreements can probably be attributed chiefly to the different analytical techniques employed.

In a study of the effect of pregnancy on the precipitable iodine of the serum, lipids were also measured in a number of cases in order to define more precisely the course of the lipids during pregnancy and also to evaluate the significance of the changes of SPI during this process.

## MATERIAL AND METHODS

The patients studied were under the supervision of members of the metabolic division of the Department of Internal Medicine. The majority were delivered in the New Haven Hospital. Blood was taken in the morning. The patients were either fasting or else had a meal containing carbohydrate, but no appreciable amounts of fat or protein (fruit juice, black coffee with sugar, toast without butter, with a little jam or jelly if desired). SPI and serum lipids were measured by methods previously described (10).

## RESULTS

Altogether 145 observations were made on 34 normal persons and 19 observations on five patients with hyperthyroidism under treatment with propylthiouracil and urea. SPI and all lipid fractions (total and free cholesterol, fatty acids and lipid phosphorus) of 31 normal subjects were measured on 114 occasions. In one instance SPI was omitted and in a number of others fractionation of lipids was incomplete.

The data from the normal subjects are presented in Table I. Although the interrelationships of the lipid fractions are not entirely unaffected by pregnancy, in general all fractions are altered synchronously and in the same direction. In every case in which repeated observations were made the lipids rise as pregnancy advances. The rise, which is extremely variable in degree, is seldom evident before 12 or more weeks have elapsed. Once it has begun, the lipids appear to increase progressively until term. After delivery they decline gradually, presumably to prepregnant concentration. The word "presumably" is used because, for obvious reasons, actual prepregnancy values are not known. There is reason to suspect that the concentrations observed in early pregnancy do not represent normal nonpregnant values. With a few exceptions, until the lapse of 12 weeks they tend to lie somewhat below the accepted average for a group of normal individuals. In a number of subjects who had more than one observation in the early weeks, cholesterol declined distinctly at this time: MMcM, C46864, MWa. The cholesterol of PG after 10 weeks of pregnancy was 212 mg. per cent; but 26 weeks after delivery it was 275 mg. per cent. Of course it may not have returned to its normal level in this interval, but it had remained at the same concentration for 15 weeks, definitely lower than it had been in the latter part of pregnancy. The first case, 43773, is particularly significant because she was studied in two pregnancies. Before the first pregnancy only lipid phosphorus was measured. Values of 9.9 and 10.2 mg. per cent were found on two occasions. It had reached practically the same value, 10.5 mg. per cent, 32 weeks after delivery. In the early weeks of both pregnancies, however, it fell to between 8 and 9 mg. per cent. As evidence that her condition did not differ appreciably on the two occasions, her lipid patterns at 17 and 18 weeks of

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[1] This investigation was supported (in part) by a research grant from the U. S. Public Health Service.

TABLE I

*Lipids in normal pregnancy*

| Case no. | Age | Weeks* | SPI | Cholesterol | | | Fatty acid | Lipid P | Neutral fat fatty acid | Lipid P − 3.7 / Free chol. |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Total | Free | Free / Total | | | | |
| | | | γ % | mg. % | mg. % | | mEq/L | mg. % | mEq/L | |
| 43773 | 29 | a 5 | | | | | | 9.9 | | |
| | | a 2 | | | | | | 10.2 | | |
| | | 7 | | | | | | 8.3 | | |
| | | 17 | | 185 | 51 | 0.27 | 12.3 | 9.6 | 3.2 | 0.116 |
| | | 30 | 7.1 | 215 | 55 | 0.25 | 14.7 | 11.0 | 4.1 | 0.133 |
| | | 33 | 5.8 | 211 | 64 | 0.30 | 14.8 | 11.3 | 4.4 | 0.119 |
| | | 39 | 6.2 | 222 | 53 | 0.24 | 15.6 | 12.0 | 4.2 | 0.157 |
| | | p 2/7 | 5.9 | 221 | 45 | 0.28 | 14.1 | 11.9 | 2.6 | 0.182 |
| | | p 12/7 | 4.4 | 217 | 69 | 0.32 | 12.2 | 11.1 | 2.0 | 0.107 |
| | | p 8 | 4.1 | 225 | 58 | 0.26 | 12.2 | 8.6 | 2.9 | 0.102 |
| | | p 32 | 5.1 | 213 | 63 | 0.30 | 13.2 | 10.5 | 3.2 | 0.108 |
| | | 9 | 4.5 | 160 | 42 | 0.26 | 10.7 | 8.8 | 2.5 | 0.121 |
| | | 13 | 4.9 | 165 | 48 | 0.29 | 9.9 | 8.6 | 1.9 | 0.102 |
| | | 18 | 5.8 | 187 | 48 | 0.26 | 12.5 | 9.9 | 3.2 | 0.129 |
| C41916 | 25 | 12 | 7.9 | | 48 | | 11.9 | 9.3 | | |
| | | 15 | 4.0 | 223 | 50 | 0.22 | 13.6 | | | |
| | | 16 | 6.2 | 234 | 60 | 0.26 | 12.8 | 10.1 | 2.4 | 0.107 |
| | | 20 | 6.6 | 243 | 70 | 0.29 | 17.7 | 10.5 | 7.1 | 0.099 |
| | | 23 | 7.0 | 263 | 77 | 0.29 | 20.7 | 11.6 | 9.2 | 0.102 |
| | | 27 | 7.3 | 316 | 94 | 0.30 | 23.4 | 14.3 | 9.6 | 0.113 |
| | | 31 | 7.0 | 342 | 100 | 0.29 | 23.9 | 14.3 | 9.3 | 0.106 |
| | | 33 | 6.9 | 332 | 90 | 0.27 | 22.5 | 13.3 | 8.5 | 0.107 |
| | | 35 | 8.0 | 355 | | | 25.1 | 14.8 | | |
| | | p 2/7 | 8.9 | 333 | 106 | 0.32 | 27.9 | 14.5 | 15.8 | 0.102 |
| | | p 6 | 5.7 | 242 | 75 | 0.31 | 12.5 | 10.3 | 2.4 | 0.089 |
| PG | 28 | 10 | 8.0 | 212 | 53 | 0.25 | 11.8 | 10.4 | 1.7 | 0.127 |
| | | 14 | 9.5 | 273 | 75 | 0.27 | 17.3 | 13.0 | 4.7 | 0.124 |
| | | 19 | 9.2 | 272 | 81 | 0.30 | 16.9 | 12.0 | 4.9 | 0.102 |
| | | 23 | 10.0 | 340 | 99 | 0.29 | 20.5 | 14.2 | 6.0 | 0.106 |
| | | 27 | 8.5 | 355 | 83 | 0.23 | 20.4 | 14.3 | 5.0 | 0.127 |
| | | 33 | 9.0 | 379 | 111 | 0.29 | 22.3 | 14.9 | 6.6 | 0.101 |
| | | 37 | 8.0 | 326 | 96 | 0.29 | 21.7 | 14.3 | 7.4 | 0.111 |
| | | p 5/7 | 7.9 | 349 | 84 | 0.24 | 16.5 | 13.2 | 1.8 | 0.113 |
| | | p 4 | 6.5 | 297 | 76 | 0.26 | 15.3 | 12.4 | 2.4 | 0.114 |
| | | p 11 | 5.0 | 271 | 75 | 0.28 | 16.3 | 12.7 | 3.8 | 0.120 |
| | | p 26 | 7.0 | 275 | 76 | 0.28 | 14.7 | | | |
| DM | 28 | 9 | 6.5 | 157 | 40 | 0.26 | 10.5 | | | |
| | | 13 | 7.1 | 156 | | | 10.6 | | | |
| | | 22 | 7.1 | 185 | 52 | 0.28 | 12.8 | 9.3 | 4.0 | 0.107 |
| | | 27 | 7.3 | 179 | 49 | 0.28 | 14.4 | 8.9 | 5.8 | 0.106 |
| | | 32 | 8.4 | 178 | | | 17.9 | | | |
| | | p 3/7 | 7.4 | 181 | 57 | 0.31 | 14.8 | 9.4 | 1.6 | 0.100 |
| | | p 6 | 4.7 | 146 | | | | 9.7 | | |
| MMcM | 20 | 4 | 4.9 | 172 | 47 | 0.27 | 11.6 | 8.8 | 3.3 | 0.109 |
| | | 8 | 4.9 | 132 | 29 | (0.22)† | 8.3 | 6.9 | 1.6 | 0.114 |
| | | 13 | 6.5 | 149 | 37 | 0.25 | 8.3 | 7.9 | 0.8 | 0.113 |
| | | 20 | 8.9 | 156 | | | 11.9 | 9.1 | | |
| | | 23 | 7.0 | 193 | 45 | 0.23 | 16.1 | 9.5 | 6.8 | 0.129 |
| | | 27 | 5.4 | 197 | 56 | 0.28 | 18.0 | 9.8 | 8.6 | 0.109 |
| | | p 6/7 | 9.4 | 233 | 64 | 0.27 | 13.7 | 10.1 | 3.4 | 0.100 |
| | | p 3 | 6.4 | 201 | 58 | 0.29 | 11.9 | 9.8 | 1.5 | 0.105 |
| | | p 8 | 5.5 | 222 | | | 13.1 | 8.0 | | |
| C46864 | 20 | 7 | 5.5 | 176 | 45 | 0.26 | 10.5 | 9.0 | 1.9 | 0.118 |
| | | 8 | 6.9 | 173 | 46 | 0.27 | 9.0 | 8.6 | 0.7 | 0.107 |
| | | 9 | 6.3 | 160 | 44 | 0.27 | 10.1 | 7.9 | 2.5 | 0.095 |
| | | 12 | 6.9 | 166 | 51 | 0.31 | 10.3 | 7.7 | 2.8 | 0.075 |
| | | 16 | 8.7 | 183 | 50 | 0.28 | 14.0 | 8.9 | 5.4 | 0.104 |
| | | 22 | 9.8 | 219 | 63 | 0.29 | 16.3 | 11.9 | 5.3 | 0.130 |
| | | 26 | 9.4 | 227 | 65 | 0.29 | 14.8 | 10.7 | 4.4 | 0.108 |
| | | 31 | 8.4 | 246 | 72 | 0.29 | 16.4 | 11.3 | 5.3 | 0.106 |
| | | 36 | 7.3 | 270 | 78 | 0.29 | 18.6 | 11.8 | 6.8 | 0.104 |
| | | 38 | 8.9 | 252 | 71 | 0.28 | 16.4 | 11.9 | 4.8 | 0.115 |
| | | 40‡ | 8.6 | 265 | 73 | 0.28 | 20.7 | 13.8 | 7.7 | 0.138 |
| | | p 4/7 | 11.2 | 277 | 80 | 0.29 | 18.2 | 14.0 | 4.8 | 0.133 |
| | | p 8 | 5.1 | 211 | 52 | 0.25 | 11.6 | 10.4 | 0.2 | 0.129 |

TABLE I—*Continued*

| Case no. | Age | Weeks* | SPI | Cholesterol |  |  | Fatty acid | Lipid P | Neutral fat fatty acid | Lipid P − 3.7 / Free chol. |
|---|---|---|---|---|---|---|---|---|---|---|
|  |  |  |  | Total | Free | Free / Total |  |  |  |  |
|  |  |  | γ % | mg. % | mg. % |  | mEq/L | mg. % | mEq/L |  |
| B85841 |  | 12 | 6.7 | 183 | 54 | 0.29 | 12.0 | 9.9 | 3.0 | 0.114 |
|  |  | 17 | 7.8 | 190 | 49 | 0.26 | 13.2 | 10.4 | 3.5 | 0.136 |
|  |  | 20 | 6.9 | 212 | 63 | 0.30 | 13.1 |  |  |  |
|  |  | 24 | 7.4 | 239 | 70 | 0.29 | 15.7 | 11.8 | 2.7 | 0.116 |
|  |  | 28 | 7.1 | 242 | 74 | 0.31 | 18.2 | 11.0 | 7.4 | 0.099 |
|  |  | *32* | *7.1* | *242* | *80* | *0.33* | *19.5* | *12.7* | *7.9* | *0.112* |
|  |  | 35 | 9.5 | 243 | 74 | 0.30 | 19.3 | 12.5 | 7.6 | 0.118 |
|  |  | 36 | 7.7 | 260 | 86 | 0.33 | 19.8 |  |  |  |
|  |  | p 1 | 8.6 | 244 | 76 | 0.31 | 16.4 | 11.5 | 5.3 | 0.102 |
|  |  | p 6 | 4.8 | 235 | 65 | 0.28 | 15.8 | 10.7 | 5.2 | 0.108 |
| B78510‖ | 29 | 16 | 7.3 | 244 | 66 | 0.27 | 14.6 | 12.3 | 2.9 | 0.130 |
|  |  | 21 | 6.4 | 238 | 89 | 0.37 | 18.5 | 12.4 | 7.4 | 0.098 |
|  |  | 25 | 6.3 | 333 | 113 | 0.33 | 21.9 | 16.0 | 7.0 | 0.109 |
|  |  | 31 | 7.4 | 350 | 144 | 0.41 | 23.0 | 16.4 | 8.1 | 0.088 |
|  |  | 35 | 6.0 | 346 | 111 | 0.32 | 22.9 | 14.3 | 8.5 | 0.096 |
|  |  | 39 | 8.8 | 423 | 111 | 0.26 | 24.9 | 15.0 | 8.1 | 0.102 |
|  |  | p 2/7 | 9.4 | 385 | 112 | 0.29 | 22.4 | 15.0 | 6.6 | 0.101 |
|  |  | p 11/7 | 7.3 | 385 | 109 | 0.28 | 18.4 | 14.9 | 2.5 | 0.103 |
|  |  | p 5 | 5.0 | 354 | 108 | 0.30 | 18.2 | 12.6 | 4.5 | 0.082 |
|  |  | p 12 | 4.5 | 292 | 80 | 0.27 | 14.8 | 11.0 | 2.9 | 0.091 |
|  |  | p 21 | 5.4 | 261 | 71 | 0.27 | 13.9 | 10.9 | 2.7 | 0.101 |
| C52217 | 32 | 5 | 8.0 | 160 | 49 | 0.31 | 10.0 | 7.6 | 0.1 | 0.080 |
| HC |  | 12 | 8.3 | 168 | 45 | 0.27 | 9.3 | 9.8 | 0.4 | 0.135 |
|  |  | 24 | 7.3 | 215 | 56 | 0.26 | 12.7 | 11.1 | 2.2 | 0.132 |
| MD | 22 | 8 | 8.6 | 176 |  |  | 10.7 |  |  |  |
|  |  | p 2/7 | 6.7 | 264 | 82 | 0.31 | 18.2 | 13.5 | 6.2 | 0.120 |
| B53121 | 37 | 6 | 4.6 | 205 | 64 | 0.31 | 12.6 |  |  |  |
|  |  | 7 | 4.4 | 230 |  |  |  |  |  |  |
|  |  | 32§ | 8.0 | 263 | 76 | 0.29 | 25.7 | 13.1 | 13.2 | 0.124 |
| VD | 34 | 36 | 8.4 | 367 |  |  | 29.8 |  |  |  |
| CF | 23 | 11 | 6.9 | 225 | 68 | 0.30 | 14.8 | 12.9 | 3.2 | 0.135 |
| C45201 | 22 | 14 | 7.6 | 213 | 60 | 0.28 | 13.5 | 10.6 | 3.4 | 0.115 |
| C13678 | 26 | 3 | 4.0 | 108 |  |  | 6.4 |  |  |  |
| AH |  | 10 | 7.6 | 197 | 62 | 0.32 | 11.1 | 8.8 | 2.5 | 0.082 |
| C42461 | 23 | 5 | 6.0 | 143 | 39 | 0.27 | 8.1 | 7.8 | 0.9 | 0.106 |
|  |  | 7 | 6.6 | 141 | 38 | 0.27 | 8.2 | 5.8 | 2.4 | 0.068 |
|  |  | 13 | 9.0 | 141 | 31 | 0.22 | 7.4 | 6.1 | 1.0 | 0.077 |
|  |  | 26 | 9.0 | 223 | 58 | 0.26 | 12.8 | 9.2 | 3.2 | 0.095 |
| C15296 | 27 | 34 | 4.8 | 329 | 94 | 0.29 | 24.6 | 15.5 | 9.5 | 0.125 |
|  |  | 40 | 4.8 | 266 |  |  | 31.5 |  |  |  |
| GJ | 28 | 20 | 7.8 | 235 | 78 | 0.33 | 19.4 | 15.4 | 6.4 | 0.150 |
| PJ |  | 14 | 6.4 | 161 | 51 | 0.32 |  |  |  |  |
| B95180 | 29 | 32 | 6.5 | 252 | 79 | 0.31 | 19.2 | 13.8 | 6.7 | 0.128 |
| MMcN |  | 32 | 6.3 | 292 | 79 | 0.27 | 19.9 | 13.8 | 6.4 | 0.128 |
|  |  | 37 | 6.6 | 296 |  |  |  |  |  |  |
| SO |  | 8 | 4.3 | 201 | 59 | 0.29 | 12.9 |  |  |  |
| C41546 | 21 | 8 | 6.2 | 201 | 66 | 0.33 | 10.7 | 8.5 | 2.3 | 0.073 |
| B769 | 29 | 12 | 7.0 | 225 | 60 | 0.27 | 11.4 | 9.9 | 1.4 | 0.105 |
| MWa | 28 | 7 | 7.6 | 146 | 38 | 0.26 | 9.6 | 6.3 | 3.1 | 0.068 |
|  |  | 11 | 7.7 | 130 | 38 | 0.29 | 9.5 | 7.4 | 2.8 | 0.074 |

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* Weeks after last menstrual period; a represents weeks before pregnancy; p represents weeks after delivery.

† Parentheses indicate that duplicate values for total or free cholesterol did not check with sufficient accuracy; therefore the ratios are not precise.

‡ One day before delivery.

§ One day before premature delivery. For details of case see (12).

‖ Received 0.02 gm. of stilbestrol daily from the end of the 16th week until delivery.

the two pregnancies respectively were almost identical. In the second pregnancy the early reduction of lipids involves all fractions proportionally. All these considerations together suggest that the lipids diminish in the early part of pregnancy. If this is the case the data tend to exaggerate the extent of the rise of lipids during pregnancy. This could also be inferred from the fact that the highest values observed in this series seldom exceed the normal limits. In only four subjects were concentrations of cholesterol greater than 300 mg. per cent encountered. The rise, nevertheless, appears to be authentic. The general range of lipids in the later part of pregnancy is distinctly above the expectation for a normal group of nonpregnant women. In addition, with the exception of 43773 and MMcM, who was followed for only eight weeks *post partum,* in those who were followed after delivery, lipids declined. The rises in these two cases were comparatively small. The rate of decline of the lipids is highly variable. There are not sufficient observations to define its course precisely. It apparently does not usually begin until a week or two have elapsed and may not be complete after as long an interval as 12 weeks.

In Table II are a few subjects who received thyroid although they had no evidences of hypothy-

TABLE II

*Serum lipids of patients who, although they had no disorder of thyroid function, received thyroid*

| Case no. | Age | Weeks* | SPI | Cholesterol | | | Fatty acid | Lipid P | Neutral fat fatty acid | Lipid P −3.7 / Free chol. | Thyroid† | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Total | Free | Free / Total | | | | | | |
| | | | *γ %* | *mg. %* | *mg. %* | | *mEq/L* | *mg. %* | *mEq/L* | | *gms,* | *wks.* |
| HL | 42 | 26 | 7.6 | 236 | 65 | 0.28 | 17.3 | 11.8 | 6.1 | 0.125 | | |
| | | 31 | 5.5 | 235 | 63 | 0.27 | | | | | 0.12 | 3 |
| | | 32 | 4.8 | 183 | 72 | (0.39) ‡ | | | | | 0.12 | 4 |
| GM | 31 | 4 | 5.2 | 167 | 47 | 0.28 | 10.2 | | | | | |
| | | 12 | 5.9 | 160 | 40 | 0.25 | 10.4 | 9.3 | 1.9 | 0.140 | 0.12 | 8 |
| HP | 35 | 7 | 6.3 | 160 | 83 | 0.52 | 9.5 | 9.7 | 1.9 | 0.072 | | |
| | | 9 | 9.9 | 188 | 49 | 0.26 | 12.2 | 10.6 | 2.5 | 0.142 | 0.18 | 2 |
| ES | 36 | 7 | 3.9 | 188 | 52 | 0.28 | 10.9 | 10.6 | 1.3 | 0.133 | | |
| | | 11 | 5.8 | 187 | 49 | 0.26 | 10.0 | | | | 0.12 | 4 |
| | | 15 | 7.4 | 169 | 47 | 0.28 | 11.0 | 9.3 | 2.4 | 0.119 | 0.24 | 3 |
| | | 27 | 5.3 | 218 | 62 | 0.28 | 14.4 | 11.6 | 3.6 | 0.127 | 0.24 | 15 |
| | | 35 | 5.4 | 255 | 71 | 0.28 | 16.5 | 11.8 | 4.9 | 0.114 | 0.24 | 23 |
| A63436 | 35 | 5 | 3.5 | 194 | 47 | 0.24 | 13.6 | 10.1 | 3.9 | 0.136 | 0.24 | 1 |
| | | 6 | 5.9 | 172 | 49 | 0.28 | 12.2 | 9.6 | 3.4 | 0.120 | 0.30 | 2 |
| | | 12 | 8.5 | 169 | 35 | 0.21 | 11.4 | 10.1 | 2.0 | 0.183 | 0.30 | 8 |
| | | 16 | 7.9 | 253 | 70 | 0.28 | 17.4 | 12.5 | 5.3 | 0.127 | 0.30 | 12 |
| | | 18 | 8.1 | 221 | 62 | 0.28 | 17.2 | 11.8 | 6.3 | 0.130 | 0.42 | 2 |
| | | 21 | 8.6 | 252 | 73 | 0.28 | 18.8 | 12.9 | 6.7 | 0.126 | 0.42 | 5 |
| | | 23§ | 8.4 | 241 | 70 | 0.29 | 14.2 | 11.8 | 3.0 | 0.115 | 0.42 | 7 |
| MW | 22 | 1 | 4.2 | 236 | 60 | 0.25 | 14.4 | 10.0 | 3.5 | 0.128 | | |
| | | 5 | 5.5 | 202 | 56 | 0.28 | 11.6 | 9.0 | 2.6 | 0.095 | | |
| | | 9 | 7.2 | 165 | 49 | 0.30 | 8.8 | 8.0 | 1.2 | 0.088 | 0.18 | 3 |
| | | 11 | 7.3 | 174 | | | 9.8 | | | | 0.18 | 5 |
| | | 15 | 7.8 | 190 | 56 | 0.29 | 12.0 | 9.1 | 3.2 | 0.096 | 0.18 | 9 |
| | | 20 | 9.4 | 228 | | | 16.4 | | | | 0.18 | 14 |
| | | 25 | 7.7 | 252 | 73 | 0.29 | 17.0 | 11.9 | 5.5 | 0.111 | 0.18 | 19 |
| | | p 2/7 | 9.7 | 259 | 77 | 0.30 | 18.2 | | | | 0 | 2/7 |
| | | p 15 | 4.1 | 233 | 70 | 0.30 | 12.3 | 10.5 | 2.3 | 0.090 | 0 | 15 |
| TK | 30 | 18 | 6.2 | 279 | 77 | 0.39 | 16.3 | 12.8 | 3.7 | 0.118 | | |
| | | 23 | 6.8 | 246 | 73 | 0.34 | 19.7 | 13.1 | 7.3 | 0.129 | 0.24 | 4 |

* Weeks after last menstrual period; p represents weeks after delivery.

† The number of weeks that the stated dose of thyroid had been taken before the determination.

‡ Parentheses indicate that duplicate values for total cholesterol did not check with sufficient accuracy; therefore the ratios are not precise.

§ On the day of miscarriage. For details of case see (12).

roidism. The general course of the lipids in these cases is not distinguished from that of the women who did not receive thyroid. This bears out the impression, expressed earlier (11, 12) that the pregnant woman tolerates or utilizes thyroid with facility. The lipids may be temporarily depressed after institution of thyroid therapy or an increase of the dose, but rise again shortly. This is evident in ES, A63436 and MW. Observations were not continued long enough in HL and TK to ascertain whether the drops were transitory or not.

Free cholesterol followed the course of total cholesterol quite closely. The ratio, free: total cholesterol, in the serum of normal persons and patients with disorders of the thyroid (10) does not depart from the narrow limits, 0.24 to 0.32. In 138 observations in this study the ratio exceeded 0.32 only 11 times and 0.33 only seven times. One of these, in HL (Table II), must be discounted because the analysis was not satisfactory. In TK (Table II) the chloresterol ratio was 0.39 after 18 weeks, 0.34 after 23 weeks. No disorder was found to explain these abnormal ratios. The pregnancy proceeded to a normal delivery. In HP (Table II) at the end of seven weeks the ratio was 0.52, but two weeks later it was only 0.26. A ratio of 0.34 was found in 98175 (Table III) two days after delivery. Ratios of 0.37, 0.33 and 0.41 were found in B78510 after 21, 25 and 31 weeks, respectively. This subject had normal ratios after 16 and 31 weeks and thereafter on seven different occasions, the last 21 weeks after delivery. This is the woman whose total cholesterol rose highest during pregnancy, reaching 423 mg. per cent at the end of 39 weeks. There was, however, no correlation between total cholesterol and the cholesterol ratio. The latter had fallen to 0.26 when the former reached its peak. The subject had had infectious hepatitis three years earlier, but had no demonstrable residua of this disease. She received stilbestrol throughout her pregnancy, being the only subject thus treated. However, the ratio returned to normal while she was still receiving stilbestrol, while the lipids remained high after it was discontinued. The ratio was less than 0.24 in

TABLE III

*Serum lipids of patients with disorders of the thyroid*

| Case no. | Age | Weeks* | SPI | BEI | Cholesterol | | | Fatty acid | Lipid P | Neutral fat fatty acid | $\frac{\text{Lipid P}-3.7}{\text{Free chol.}}$ | Lugol's solution | Propylthiouracil | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Total | Free | $\frac{\text{Free}}{\text{Total}}$ | | | | | | | |
| | | | γ % | γ % | *mg. %* | *mg. %* | | *mEq/L* | *mg. %* | *mEq/L* | | *gtts.* | *mg.* | *wks.*† |
| C24644 | 34 | 6 | 9.6 | | 182 | 47 | 0.26 | 10.6 | 8.3 | 2.2 | 0.098 | 12 | 100 | 25 |
| | | 14 | 11.3 | | 253 | 74 | 0.29 | 16.3 | 11.9 | 4.8 | 0.111 | 12 | 75 | 7 |
| | | 20 | 9.1 | | 252 | 77 | 0.31 | 16.4 | 11.9 | | | 12 | 75 | 13 |
| | | 30 | 8.9 | 7.2 | 276 | 86 | 0.31 | 20.2 | 13.9 | 7.2 | 0.119 | 12 | 75 | 23 |
| | | p 1/7 | 9.1 | 7.6 | | | | | | | | | | |
| | | p 5 | 7.7 | 4.7 | 243 | | | 19.5 | 13.1 | | | 12 | 100 | 5 |
| 98175 | 32 | 10 | 9.4 | 6.9 | 257 | 73 | 0.28 | 16.8 | 10.4 | 6.0 | 0.072 | 15 | 450 | 6 |
| | | 14 | 14.9 | 13.7 | 242 | 75 | 0.31 | 18.0 | 11.7 | 6.9 | 0.107 | 15 | 450 | 10 |
| | | 21 | 8.6 | 6.4 | 342 | 94 | 0.28 | 24.6 | 15.3 | 9.3 | 0.099 | 15 | 500 | 7 |
| | | 24 | | 14.0 | 278 | 78 | 0.28 | 25.3 | | | | 15 | 300 | 3 |
| | | 26 | 14.9 | 12.9 | 318 | | | 25.2 | 14.6 | | | 15 | 300 | 5 |
| | | p 2/7 | | 16.4 | 305 | 105 | 0.34 | 22.7 | 14.9 | 8.9 | 0.107 | 15 | 150 | 8 |
| A25444 | 26 | 18 | 11.2 | 6.7 | 200 | 59 | 0.29 | 16.2 | 13.4 | 4.8 | 0.164 | 7 | 300 | 8 |
| | | 23 | 8.6 | 6.1 | 349 | 73 | 0.21 | 19.1 | 14.0 | 3.8 | 0.141 | 7 | 300 | 13 |
| | | 28 | | 11.8 | 159 | 49 | 0.31 | 12.5 | | | | 7 | 150 | 5 |
| B94697 | 36 | 37 | 8.7 | | 186 | 54 | 0.29 | 19.9 | 11.5 | 10.8 | 0.144 | 10 | 50‡ | 16 |
| | | 40 | 11.3 | | 189 | 59 | 0.31 | 20.2 | 10.9 | 10.5 | 0.122 | 10 | 25‡ | 3 |
| | | p 2/7 | 10.5 | | 173 | | | 17.4 | | | | 10 | 25‡ | 3 |
| | | p 2 | 11.1 | 6.8 | 270 | 72 | 0.27 | 21.1 | 11.6 | 9.2 | 0.110 | 10 | 25‡ | 5 |
| C19922 | 36 | 19 | 9.2 | | 179 | 48 | 0.27 | 15.2 | | | | 14 | 300 | 14 |

* Weeks after last menstrual period; p represents weeks after delivery.
† Number of weeks patients had been taking propylthiouracil.
‡ Thiourea instead of propylthiouracil.

three subjects. One of these can be attributed to analytical error. No cause for abnormally low ratios has been discovered. In no case could variations of the ratio, within or outside of the normal range, be related to the stage of pregnancy or to other characteristics of the lipids. The deviations from the normal range must, therefore, be attributed to adventitious disorders, the nature of which was not discovered.

In normal persons the relationship of phospholipid to cholesterol is most precisely defined by the equation, $\frac{\text{mg. per cent of lipid P} - 3.7}{\text{mg. per cent of free cholesterol}} = 0.106$ (13). In the present study the average ratio was 0.113, varying from 0.067 to 0.182. No significance can be attached to the slightly elevated average ratio or the variability of the ratio. These features could not be correlated with the course of pregnancy, other lipid fractions or the cholesterol ratio.

The most distinctive feature of the lipid pattern is an increase of the concentration of neutral fat as pregnancy progresses. Although this is usually moderate and varies from case to case, it is unmistakable and consistent in all subjects and therefore significant. Neutral fat and cholesterol appear to be roughly correlated; but the correlation probably derives only from the fact that both rise as pregnancy progresses. Neither in individual cases nor in the series as a whole do the two functions vary proportionally. Neutral fat appears to fall earlier than cholesterol does after delivery.

Both SPI and cholesterol rise as pregnancy advances. There is no reason except this association to connect the two phenomena. In most other circumstances cholesterol tends to diminish as SPI rises.

In Table III are five pregnant women with hyperthyroidism who were treated with this drug and Lugol's solution of iodine. Although the lipids are more variable in these patients than they are in normal women they have the same tendency to rise as pregnancy advances and to decline after delivery. SPI in these cases does not mirror precisely the degree of thyroid activity for reasons pointed out elsewhere. SPI may rise further in hyperthyroid than in normal persons during pregnancy (12). In addition administration of iodine causes spurious increases of SPI (14). Butanol-extractable iodine (BEI), figures for which are given in several instances, is a better measure of thyroid activity in these cases. In A25444 and 98175 the lipids fell when signs of frank hyperthyroidism appeared with BEI's above 10 gamma per cent. In the latter case, they rose again in spite of the continued hyperthyroidism.

## DISCUSSION

There is little to add to this description. The causes of these variations in the concentrations and interrelationships during pregnancy will remain conjectural until the general metabolism of pregnancy has been further elucidated. The early decline of lipids, if it is verified, may be related to vomiting of pregnancy, but this was not conspicuous in 43773 (Table I), whose lipids fell with the onset of both pregnancies. It has been shown that certain estrogenic materials increase the lipids of the serum and livers of rats and guinea pigs (15–17). Increases of neutral fat are reported to occur either after meals or when fat is being mobilized rapidly from the depots to the liver for formation of ketone bodies. It is intriguing to speculate about the possible connection of this phenomenon in pregnancy with the recognized tendency of the pregnant woman to spend carbohydrate prodigally and her unusual susceptibility to ketosis. Variability of the decline of lipids after delivery may be connected with lactation; but no such connection could be discerned in this small series of subjects. If the hyperlipemia were related to the great discharge of estrogenic materials that characterizes pregnancy such a delay should not be expected.

The association of hyperlipemia with high SPI might be interpreted as evidence of relative hypothyroidism. It is conceivable that the demands of the tissues for the thyroid hormone and utilization of the hormone are augmented during pregnancy and that the increased discharge of the hormone manifested in the elevated SPI is not sufficient to meet these demands. This is an unlikely explanation for two reasons. When the hyperthyroid patient, 98175, lapsed into overt hyperthyroidism, although the lipids fell they still remained far higher than they would have been in a hyperthyroid nonpregnant woman. Moreover in the hyperlipemia of hypothyroidism neutral fat does not rise (10).

## SUMMARY AND CONCLUSIONS

The lipids and precipitable iodine (SPI) of the serum of 34 normal women and five women with medically treated hyperthyroidism have been measured at intervals during pregnancy and after delivery. There is evidence to suggest that the lipids may decline slightly in the early weeks of pregnancy. After about the 12th week, however, they rise progressively until delivery, thereafter declining at a variable rate. In this rise total and free cholesterol and phospholipids participate proportionally, maintaining their normal relations to one another. Neutral fat, however, rises proportionally far more than the other lipid fractions and declines more rapidly than the others after delivery.

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# THE BIOLOGICAL ACTIVITY OF A FLAVONOID (VITAMIN "P") COMPOUND[1, 2]

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*(Submitted for publication June 9, 1950; accepted, January 17, 1951)*

The elaboration of a practical and reliable biological test for the estimation of vitamin "P" activity is the most urgent and important problem with which every investigator in this field is confronted. The complexity of the physiological activity of flavonoid compounds was responsible for the obstacles in finding a satisfactory solution of this problem. The attempts to induce experimental avitaminosis "P" gave contradictory observations making this test unreliable for any practical use (1–8). A method was suggested for the estimation of vitamin "P" activity by using the capillary bed in the rat-mesoappendix, with a compound topically applied. It was found, however, that most of the flavonoid compounds, except catechins, did not produce any vasoconstrictor effect (9, 10). Considerable work was done with the determination of capillary permeability by using chloroform wheals applied to the skin and simultaneous injections of trypan blue. This test pertaining rather to permeability changes than to increased capillary fragility has demonstrated a great variation in the response of experimental animals to the same flavonoid compound (11, 12). More recently it was suggested that radiation injury to the capillary wall might serve as a basis for the estimation of vitamin "P" activity. Purpura, for which ascorbic acid has proved ineffective, has been a constant feature of radiation illness. It has been shown that some flavonoid compounds exert protective action against radiation injury to the capillary system (13–18).

In our present report which covers both experimental and clinical data particular attention was paid to the determination of vitamin "P" activity in the condition where increased capillary fragility rather than permeability changes were evidenced.

## MATERIAL

A flavonoid compound isolated from citrus fruit was used for our experimental and clinical work. It contained four identified factors; namely, hesperidin-chalcone, glucose-hesperidin chalcone, eriodictin and quercitrin-like substance, which seemed to form a complex molecule as U.V. readings have shown. It was a yellow powder slightly hygroscopic, with impurity of 0.6%, mostly calcium and phosphorus, soluble in water at the pH 7.0 or in alkaline medium. It was tested by the technique of Ambrose-DeEds (11) by the application of chloroform to the skin of rabbits by means of a cotton-tipped applicator. We used a dose of 10 mg. per kilogram of weight of the flavonoid compound injected subcutaneously 20 minutes before the injection of trypan blue (10 cc.) in the marginal ear vein and the application of chloroform wheal to the skin. The average time of the appearance of the dye for 20 rabbits was 42 minutes 54 seconds as against one minute 57 seconds in the control batch of 10 rabbits. These figures when compared with those obtained by Ambrose-DeEds with rutin (three minutes for control and 15.7 minutes for rutin treated rabbits) seem to indicate a higher biological activity of the citrus flavonoid compound than that of rutin.

## EXPERIMENTAL

### 1. *Increased capillary fragility induced by leukotaxine*

Menkin (19, 20) found that an inflammatory exudate induces an increase in capillary permeability. This was manifested by the accumulation of trypan blue from the circulation in a cutaneous area previously injected with an exudate. This effect was likewise duplicated by leukotaxine, a nitrogenous substance extracted from inflammatory exudation. The effect of leukotaxine on increased capillary permeability is wholly or in part inhibited by the presence of an extract of the adrenal cortex. Hyman and Chambers (21) also found that adrenal cortical extracts are extremely efficient in reducing capillary permeability in perfused isolated limbs. According to them, the cortical extracts have no dilator or constrictor action and presumably affect the capillary wall directly by decreasing the pore size of the intercellular cement.

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[1] Presented to the Sixth International Congress of Radiology, Section of Radiobiology, London, July 27, 1950.

[2] This work was aided by grants from The Marcia Tucker Foundation and the U. S. Vitamin Co., New York.

TABLE I

*Inhibitory effect of the flavonoid compound on induced capillary permeability*

Rabbits Nos. 37–46

| Rabbits | Treatment | Appearance of dye after injection in | | |
|---|---|---|---|---|
| | | 15 min. | 20 min. | 30 min. |
| R. 37, f. | Flavonoid plus 3 mg. leukotaxine | 0 | 0 | traces |
| R. 38, m. | | 0 | 0 | 0 |
| R. 39, m. | | 0 | 0 | + |
| R. 40, m. | | 0 | 0 | 0 |
| R. 41, m. | | 0 | traces | traces |
| R. 42, m. | | 0 | traces | + |
| R. 43, m. | | 0 | 0 | 0 |
| R. 44, m. | | 0 | traces | + |
| *Controls* | | | | |
| R. 45, m. | No flavonoid and 3 mg. leukotaxine | + | ++ | ++ |
| R. 46, m. | | + | ++ | +++ |

N.B. Diet of rabbits was Purina rabbit chow checkers. Ingredients of Purina rabbit chow: Ground oats, corn meal, soybean oil meal, linseed oil meal, corn germ meal, dehydrated alfalfa meal, wheat gray middlings, animal protein factor supplement, riboflavin supplement, D activated plant sterol, 2% defluorinated phosphate, .5% iodized salt.

In our experiments we have studied the effect of the flavonoid compound upon the capillary changes induced by leukotaxine using Menkin's technique for indicating these changes. Rabbits of an average weight of 2 kilograms were given 10 mg./kilo/weight of the flavonoid compound 20 minutes before subcutaneous injection of 3 mg. of leukotaxine. Immediately afterwards 12 cc. of 1% trypan blue in saline solution were introduced in the marginal ear vein. The change in capillary permeability was gauged by the degree of dye accumulation in the various skin areas. Altogether 10 rabbits were used for these experiments. The flavonoid compound was injected subcutaneously 10 mg./kilo/weight, shortly before the injections of trypan blue. In Table I the results are summarized.

It appears that the citrus flavonoid compound decreases capillary permeability induced by leukotaxine. Its effect is similar in this respect to that of adrenal cortical extracts, as compared with the data reported by Menkin.

### 2. *Increased capillary fragility induced by bacterial polysaccharide*

The polysaccharide isolated by M. J. Shear from *Serratia marcescens* produces an extensive hemorrhage in the tumors of animals and, when the dose is sufficiently large, can even cause death (22, 23). The striking fact is that normal rats and mice are much less susceptible to the toxic effect of this compound and can survive a much larger dose than can the cancerous animals. Four or five hours after the bacterial polysaccharide is injected into the cancerous animals, profuse hemorrhages are also observed in the adrenal gland. When adrenal cortical extract is employed in combination with polysaccharide, tumor breakdown is delayed (instead of in six to seven hours it might occur in 24 hours) and the effect on the adrenal gland is minimized (24).

For our experiments we used August Rat Carcinoma (Crocker Laboratory, Columbia University), a fast growing tumor. As a rule only tumors averaging in size 8–9 cm$^3$. were used for experimentation. When a rat bearing such a tumor is given 0.5 mg./100 g./weight of a bacterial polysaccharide (P–25) which we received from Dr. Shear, death occurred in about seven hours preceded by profuse hemorrhage in the tumor. Histological examination of the tumor, performed every

TABLE II

*Inhibitory effect of the flavonoid compound on induced capillary fragility of tumors (bacterial polysaccharide)*

| Rat Nos. | Treatment | | Result: death or survival |
|---|---|---|---|
| | P-25* | Flavonoid | |
| 171-A, m. | 0.5 mg. | 3 mg. | Death in 17 hrs. |
| 171-B, f. | 0.5 mg. | 3 mg. | Death in 22 hrs. 25 min. |
| 171-C, m. | 0.5 mg. | 3 mg. | Death in 18 hrs. |
| 171-D, m. | 0.5 mg. | 3 mg. | Death in 19 hrs. 10 min. |
| 171-E, m. | 0.5 mg. | 3 mg. | Death in 20 hrs. 40 min. |
| 174-A, f. | 0.5 mg. | 3 mg. | Death in 19 hrs. |
| 174-B, m. | 0.45 mg. | 3 mg. | Death in 24 hrs. 30 min. |
| 174-C, m. | 0.5 mg. | 10 mg. | Death in 36 hrs. |
| 174-D, m. | 0.5 mg. | 10 mg. | Survived |
| 174-E, m. | 0.5 mg. | 10 mg. | Death in 52 hrs. |
| 174-F, m. | 0.5 mg. | 10 mg. | Survived |
| 177-A, f. | 0.5 mg. | 10 mg. | Survived |
| 177-B, m. | 0.5 mg. | 10 mg. | Death in 66 hrs. |
| 177-C, f. | 0.5 mg. | 10 mg. | Survived |
| 177-D, m. | 0.4 mg. | 10 mg. | Survived |
| 177-E, m. | 0.45 mg. | 10 mg. | Survived |
| 177-F, m. | 0.5 mg. | 10 mg. | Survived |
| 177-H, m. | 0.5 mg. | 10 mg. | Death in 26 hrs. |
| 178-A, m. | 0.5 mg. | 10 mg. | Survived |
| *Controls* | | | |
| 178-B, m. | 0.5 mg. | None | Death in 6 hrs. 25 min. |
| 178-C, f. | 0.5 mg. | None | Death in 7 hrs. 35 min. |
| 178-D, m. | 0.5 mg. | None | Death in 9 hrs. |
| 178-E, m. | 0.5 mg. | None | Death in 7 hrs. 30 min. |
| 178-F, f. | 0.5 mg. | None | Death in 8 hrs. 20 min. |
| 178-H, m. | 0.5 mg. | None | Death in 7 hrs. 15 min. |

* P-25 is a preparation of Shear bacterial polysaccharide.

TABLE III

*Effect of flavonoid compound on survival of irradiated rats*

*A. Control group of 40 rats (no flavonoid)*
*Initial average weight: 183 g.*

| Number of days of survival: | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Number of rats succumbed: | 1 | 3 | 3 | 2 | 3 | 3 | 3 | 3 | 2 | 4 | 2 | 1 | 2 | = 32 |
| Mortality rate: | 80% | | | | | | | | | | | | | |

*B. 20 rats given 4 mg. flavonoid for 10 days*
*Initial average weight: 186 g.*

| Number of days of survival: | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | |
|---|---|---|---|---|---|---|---|---|---|---|
| Number of rats succumbed: | 1 | 1 | 2 | 0 | 0 | 1 | 1 | 1 | 1 | = 8 |
| Mortality rate: | 40% | | | | | | | | | |

*C. 40 rats given 5 mg. flavonoid for 30 days*
*Initial average weight: 175 g.*

| Number of days of survival: | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | |
|---|---|---|---|---|---|---|---|---|---|---|
| Number of rats succumbed: | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | = 4 |
| Mortality rate: | 10% | | | | | | | | | |

N.B. Basal diet of rats was Purina laboratory chow. Ingredients of basal diet: Meat meal, dried skim milk, wheat germ, fish meal, liver meal, dried beet pulp, corn grits, oat middlings, soybean meal, dehydrated alfalfa meal, molasses, animal protein factor supplement, riboflavin supplement, brewer's dried yeast, thiamin, niacin, vitamin A and D feeding oils, D activated plant sterol, 1% steamed bone meal, 0.5% iodized salt, 0.02% manganese sulfate.

hour after the injection of the compound, revealed that the capillary system of the tumor was first to respond to the toxic effect of polysaccharide by steadily increasing bleeding. There seemingly was present a gradual increase in capillary fragility. When a small dose of 3 mg./100 g./weight of the flavonoid compound was given subcutaneously, one or two hours before the injection of polysaccharide, the life of the animal was prolonged up to 18–24 hours and the capillary hemorrhages considerably delayed and less pronounced. A larger dose of the flavonoid, 5 mg./100 g./weight given twice, before and shortly after the injection of P–25, prevented in many instances the death of the animal. The hemorrhages were still present but in a relatively small area and there was a considerable delay in their appearance, in some instances 48 hours after the injection of P–25. In the group of rats receiving 3 mg./100 g./weight there was a partial breakdown of the tumor which was absent, however, in the majority of the cases receiving 10 mg./100 g./weight of the flavonoid compound.

The following Table II summarizes the results of the experiments on 25 rat bearers of tumor (19 males and six females).

There seems to be some similarity in the protective action of the flavonoid against the hemorrhage-producing effect of bacterial polysaccharide in tumor-bearing rats with that of adrenal cortical extracts. The flavonoid compound gives protection not only to the capillary system of the tumor but to the capillary system of the adrenal gland as well. Both cortex and medulla in the animals treated with polysaccharide and the flavonoid appear relatively normal with few residual hemorrhages in zone X of the cortex.

### 3. *Increased capillary fragility induced by ionizing radiation*

In our next series of experiments, radiation injury to the capillary wall was investigated. Field and Rekers (16) in their extensive investigation on dogs found that certain flavonoids gave a considerable protection against radiation, and expressed the opinion that vitamin "P" factors affect the vascular system directly. They concluded that: "previous misunderstanding of the nature of vitamin 'P' has arisen from both the failures to recognize that several flavonone analogues possess very similar anti-hemorrhagic activity and that ascorbic acid has the capacity to potentiate activity in other flavonones."

In our investigation 100 rats, 63 males and 37 females, were submitted to a total body, near-lethal dose of radiation. The average weight of the rats was 180 g., ranging from 160 to 205 g. The radiation factors were: 250 kv, with 0.5 mm. Cu and 3.0 mm. Bakelite Filters. Target distance was 27.5 cm. and 210 r/min. dose rate. All rats received 800 r total body X-radiation in a single exposure. Forty rats served as control. The mortality rate in the control group was 80%. Only eight rats survived at the end of three weeks. Of the treated animals 20 were given 4 mg. of the flavonoid compound for 10 days, three days prior

TABLE IV

*The protective action of the flavonoid compound against radiation erythema*

14 cases

| Case no. | Diagnosis | X-ray dosage | Flav. cpd. | Skin reaction |
|---|---|---|---|---|
| 1. F—38 | Carcinoma of cervix | 3,100 r skin dose to 4 pelvic fields | 600 mg. | Minimal skin reaction. Nausea and vomiting. |
| 2. M—44 | Carcinoma of gingiva | 3,000 r skin dose to 3 fields | 300 mg. | No erythema. No nausea or vomiting. |
| 3. F—61 | Carcinoma of pleura | 2,900 r to 3 thoracic fields<br>4,000 r to cerv. f. | 300 mg. | No erythema. No nausea. Slight tanning. |
| 4. F—32 | Carcinoma of gingiva | 2,600 r to 2 skin fields; 10,000 gamma | 300 mg. | No skin erythema, dry erythema of mucous. |
| 5. F—44 | Carcinoma of cervix | 4,000 r skin dose to each of 4 pelvic fields | 600 mg. | No erythema. Bleeding arrested. |
| 6. F—50 | Hepatoma | 3,000 r skin dose to each of 4 abd. portals | 300 mg. | Mild erythema. No nausea or vomiting. |
| 7. F—70 | Carcinoma of cervix | 3,000 r to each of 4 skin portals | 300 mg. | Slight nausea. No erythema. |
| 8. F—38 | Adenocarcinoma of rectum | 5,000 r to each of 4 pelvic fields | 600 mg. | No nausea. Slight skin reaction. |
| 9. M—56 | Metastatic carcinoma cervical node | 4,000 r skin dose delivered in 10 days | 300 mg. | Slight dry erythema. |
| 10. F—65 | Carcinoma of ovary | 1,800 r to each of 4 abd. fields | 300 mg. | No erythema. |
| 11. F—45 | Scirrhous carcinoma of breast | 3,400 r skin dose to each of 4 skin portals | 600 mg. | Slight tanning. Skin intact. |
| 12. F—55 | Carcinoma of cervix | 3,000 r skin dose to each of 4 pelvic portals | 600 mg. | No erythema. |
| 13. F—70 | Carcinoma of cervix | 3,100 r skin dose to each of 4 pelvic portals | 300 mg. | Mild skin reaction. |
| 14. M—58 | Lymphosarcoma of stomach | 3,200 r skin dose to each of 3 abdom. portals | 300 mg. | No erythema. |

to radiation and seven days post radiation. This dose gave a partial protection. The mortality rate was reduced 40%, with 12 rats surviving the exposure. Forty rats were given 5 mg. of the compound for 30 days, five days prior to the exposure and 25 days post radiation, with a total amount of 150 mg. In this group the mortality rate was reduced to 10% with 36 rats surviving. (Table III.)

From these observations it appears that the citrus flavonoid compound gives considerable if not complete protection to rats against a total body, near-lethal dose of radiation.

## CLINICAL

Roentgen sickness is a syndrome in which many factors seem to be involved. It was reported that vomiting and nausea can be alleviated by the use of some antibiotics (aureomycin) and the opinion was expressed that these manifestations are partially due to bacterial infections controlled by antibiotic substances (25). In our clinical studies particular attention was paid to radiation erythema and the biological activity of the flavonoid compound was measured in terms of prevention of the erythematous response. The present report covers 92 patients submitted to large doses of radiation and receiving the flavonoid compound. The preparation was administered orally, 300–500 mg./day for five days prior to the exposure and during the whole course of radiotherapy. Table IV presents the results of the investigation conducted by Dr. Isidore Arons, Department of Radiotherapy, Harlem City Hospital, New York City.

From this preliminary clinical report it appears that the flavonoid compound prevents to a considerable degree the appearance of radiation erythema, while nausea and vomiting might persist in spite of flavonoid therapy.

## DISCUSSION

All the available evidence indicates that the capillary wall in normal tissue is comparatively impermeable both to serum albumin and to serum globulin. Consequently the average effective pore size of the capillary wall must be less than six millimicrons. It has been suggested that pore size is confined by the intercellular cement lying between the endothelial cells. It is known from the work of Chambers and Zweifach and others (26, 27) "that the intercellular cement dissolves under certain physical conditions such as the absence of calcium or at slightly acid pH." J. F. Danielli pointed out that: "It is quite certain that anything which affects the physical condition of the intercellular cement affects the pore size" (28). This in turn will affect the effectiveness of the pores as "perfect sieves" and consequently induce what we know as increased capillary fragility. According to Danielli "under normal conditions associated with increased capillary permeability the walls of the pores through this cement are coated with a layer of adsorbed serum protein which effectively reduces the area of pore through which filtration may occur." Displacement of this adsorbed serum protein by other molecules of small diameter may result in an increase in pore size and in a consequent injury to the capillary wall.

This basic concept of capillary pathology indicates the necessity of making a clear distinction between increased capillary permeability and capillary fragility. The latter term should be used only in those cases where chemical lesions in the capillary wall, or more specifically in the intercellular cement, are present.

As Haden, Schneider and Underwood have shown (29) capillary injury "is by far the most frequent cause of clinical hemorrhagic disease" and this injury "may be due to infection, drugs, toxemia, allergy, or nutritional disturbances." They pointed out that increased capillary fragility is due to changes in the intercellular cement substance or to primary damage to the endothelial cells.

In our present work we have demonstrated that the flavonoid compound gave considerable protection against the toxic effect of leukotaxine, bacterial polysaccharide and ionizing radiation. In all these instances an increase in capillary fragility was prevented by the administration of this compound. Although the mechanism of action of the flavonoid compound is not as yet explained we are inclined to agree with Field and Rekers that vitamin "P" factors affect the capillary system directly, perhaps participating as a principal in the "wear and tear" of a part or all of the capillary system, inhibiting its degeneration and taking part in its regeneration, specifically as far as the intercellular cement is concerned. Seemingly vitamin "P" activity is most evident when chemical lesions in the capillary wall are present and increased capillary fragility exists.

## SUMMARY

1. A flavonoid preparation from citrus fruit and containing four identified factors was tested, experimentally and clinically, on its biological activity.

2. Ten mg./kilo/weight of this mixture administered to rabbits 20 minutes before subcutaneous injection of 3 mg. of leukotaxine wholly or in part inhibited the effect of this inflammatory exudate factor on capillary permeability.

3. Ten mg./100 g./weight of this material protected to a considerable degree the capillary system of August Rat Carcinoma and of the adrenal gland of cancerous rat against the toxic effect of 0.5 mg. of bacterial polysaccharide.

4. Five mg. of this preparation administered orally for 30 days, five days prior to the exposure and during 25 days post radiation, gave a considerable but not complete protection against a total body, near-lethal dose of X-ray radiation. The mortality rate in the group of 40 irradiated rats was reduced to 10% as against 80% of mortality in the control batch of 40 irradiated rats.

5. Ninety-two patients submitted to deep X-ray therapy and given the mixed flavonoids for five days prior to the exposure and during the whole period of radiotherapy, 300–600 mg./day, orally, manifested considerable diminution in radiation erythema of the skin and in some cases a complete absence of it. Nausea and vomiting, however, persisted in some of the treated patients.

## ACKNOWLEDGMENTS

We would like to acknowledge our appreciation to Dr. Isidore Arons and Dr. John Freeman, of Harlem City Hospital, New York City; to Dr. Theodore R. Miller, of Pack Medical Group, New York City; to Dr. C. B. Rossignol, of St. Luke's Hospital, New York City; and to Dr. F. R. Hurt, of St. Vincent Hospital, Jacksonville, Fla., for the clinical investigation they conducted with the *flavonoid compound.*

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# CARDIAC OUTPUT IN POSTURAL HYPOTENSION [1]

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(Submitted for publication May 23, 1949; accepted, February 12, 1951)

The arterial pressure is kept at a nearly constant level in normal man by simultaneous adjustments of the cardiac output and calibre of the arterioles. The normal response on motionless standing is arteriolar constriction and a moderate fall in cardiac output. The arteriolar constriction is usually sufficient to prevent a fall in mean pressure. In certain persons a marked fall in arterial pressure occurs on motionless standing. The purpose of this study is to evaluate the relative importance of an abnormal decline in cardiac output and of a failure of normal vasoconstriction in producing the fall in arterial pressure.

## METHODS

Among the 12 patients studied, postural hypotension was idiopathic in three, associated with diabetes mellitus in five, with tabes dorsalis in two, with probable syringomyelia in one, and followed bilateral sympathectomy ($T_1$ through $L_1$) for hypertension in one case.[3] All subjects were studied in the morning, usually after a light breakfast. Observations were made on a tilting table in the supine position, and at a foot-down angle which was usually 60° from the horizontal. In the latter position the patient was supported by a foot-plate. The tilted position was maintained for three to four minutes before beginning air collection for determination of oxygen consumption. The air collection usually occupied two minutes, and toward the end of this time blood samples were drawn and pressure measurements were begun. The total duration of tilt was seven to eight minutes. The time allowed for adjustment to the tilted position is therefore quite short. However, if more time is allowed to reach a steady state during the tilt, there is danger of collapse. None of these subjects showed evidence of impending collapse. In one subject (J. D.) A–V blood oxygen differences were determined three minutes apart during the tilt, and in two subjects (C. G. and J. P.) duplicate venous samples were taken approximately one minute apart (before and after drawing the arterial sample). The results, presented in Table I, do not indicate rapid shifts in A–V oxygen difference during the period of measurement.

Albumin infusion consisted of 75 gm. of human serum albumin [4] made up with normal saline to a volume of 1,300 cc. and given intravenously within a period of 15 minutes.

Cardiac outputs were determined by the Fick principle following intracardiac catheterization (1). Mixed venous blood was obtained from the atrium or the pulmonary artery; arterial blood, from the brachial artery through an inlying needle. Blood oxygen contents were determined by a spectrophotometric method (2) or by the method of Van Slyke and Plazin (3). In the latter case, duplicate analyses were required to check within 0.1 vol. %. With the spectrophotometric method, duplicate determinations are not in general done because of the simplicity of the technique. Repeated determinations of arterio-venous oxygen difference on the same blood samples yield results within 0.2 vol. %, unless the hemolyzed samples are grossly turbid. All samples are checked for turbidity before reading. Spectrophotometric oxygen determinations were made in subjects J. D., C. G., C. C., J. P., and C. B. Oxygen consumption was measured by timed collection of expired air in Douglas bags, analysis in the Haldane apparatus, and volume measurements in a Tissot spirometer. Intracardiac and arterial pressures were measured by Hamilton manometers. The point of zero reference was 5 cm. below the fourth costochondral junction in the supine position and in the two instances where measurements were made at − 30° (head down). In the upright tilts the zero point was taken at the fourth costochondral junction. Mean arterial pressures were obtained by planimetry.

"Calculated resistance" is expressed as the ratio between mean arterial pressure in mm. Hg and cardiac index in liters per minute per square meter of body surface. This is a general form for the expression of resistance (4). In the present study it is intended to serve as a rough index of over-all changes in the arteriolar system of a given subject. A rise in the function is taken to indicate over-all narrowing of the arteriolar bed, while a fall indicates dilatation. For this purpose it must be admitted that the function is quite imperfect. In the study of regional vascular changes, the general expression for resistance has been refined by corrections for yield pressure and viscosity (5). It is uncertain how much gain in reliability would be achieved by the application of these corrections in the present study. A major uncer-

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[1] This work was supported by a grant from the Life Insurance Medical Research Fund and the Anna H. Hanes Memorial Fund.

[2] U. S. Public Health Service Postdoctorate Research Fellow.

[3] This patient was studied through the courtesy of Dr. Keith Grimson, Department of Surgery, Duke University School of Medicine.

[4] Supplied by the American Red Cross.

tainty arises because comparisons are made of resistances in different body positions. The change from supine to tilted position causes a change in distribution of blood flow to the body, with a resultant change in the contribution of different vascular regions to the total resistance. Since resistance is affected by local factors such as length of small vessels and tissue pressure, and since the influence of these factors is altered by a change in distribution of flow, even the refined formula may not well reflect arteriolar changes in this particular situation. For this reason the general expression is used. Its limitations are emphasized by terming it "calculated resistance." The function is studied only as it changes with *body position in a given individual.* Direct comparisons of calculated resistance are not made between different subjects, nor are resistances compared in the same subject before and after the administration of albumin.

## RESULTS

The data are presented in Table I.[5] The last two cases in the table, G. G. and T. I., usually had

TABLE I

| Subject | Comment | Position | Atrial pressure (*mm. Hg*) | Arterial pressure (*mm. Hg*) | | | Calculated resistance (*mm. Hg/L/min./M²*) | Blood oxygen content (*vol. %*) | | | Oxygen consumption (*cc./min./M²*) | Cardiac index (*L/min./M²*) | Heart rate (*beats/min.*) | Per cent of resting value | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Systolic | Diastolic | Mean | | Arterial | Venous | A-V difference | | | | Mean arterial pressure | Calculated resistance | Cardiac index |
| J. D. | Female, 65, Idiopathic postural hypotension | Flat | 1 | 164 | 79 | 119 | 44 | 10.4 | 6.1 | 4.3 | 116 | 2.7 | 91 | | | |
| | | 50°, 2 min. | | | | | | 11.2 | 4.9 | 6.3 | | | | | | |
| | | 50°, 5 min. | −5 | 56 | 33 | 43 | 17 | 11.7 | 6.1 | 5.6 | 139 | 2.5 | 99 | 36 | 39 | 93 |
| | | −30° | 4 | 129 | 65 | 96 | 26 | 12.0 | 8.7 | 3.3 | 121 | 3.7 | 93 | 81 | 59 | 137 |
| C. G. | Male, 22, Diabetes, postural hypotension | Flat | 0 | 133 | 86 | 107 | 22 | 14.5 | 12.0<br>12.1* | 2.5 | 119 | 4.8 | 104 | | | |
| | | 60° | −4 | 86 | 51 | 64 | 19 | 14.0 | 9.5<br>9.6* | 4.5 | 149 | 3.3 | 140 | 60 | 86 | 69 |
| | | −30° | 1 | 139 | 88 | 110 | 22 | 13.8 | 10.8 | 3.0 | 148 | 4.9 | 110 | 103 | 100 | 102 |
| C. C. | Male, 60, Diabetes, postural hypotension | Flat | −4 | 191 | 94 | 130 | 32 | 19.1 | 14.4 | 4.7 | 193 | 4.1 | 113 | | | |
| | | 60° | −6 | 128 | 82 | 96 | 36 | 17.8 | 11.0 | 6.8 | 186 | 2.7 | 126 | 74 | 112 | 66 |
| J. P. | Male, 43, ?Syringomyelia, postural hypotension | Flat | 5 | 115 | 72 | 90 | 45 | 19.1 | 12.1 | 7.0 | 138 | 2.0 | 57 | | | |
| | | 60° | −3 | 73 | 55 | 60 | 35 | 19.6 | 11.6<br>11.2* | 8.2 | | 1.7† | 101 | 67 | 78 | 85 |
| C. B. | Male, 46, Diabetes, postural hypotension | Flat | 0 | 147 | 80 | 111 | 33 | 15.1 | 10.3 | 4.8 | 163 | 3.4 | 77 | | | |
| | | 60° | 0 | 120 | 76 | 92 | 30 | 15.1 | 9.8 | 5.3 | 162 | 3.1 | 103 | 83 | 91 | 91 |
| R. S. | Male, 25, Hypertension, four years post-sympathectomy | Flat | | 141 | 82 | 107 | 30 | 19.3 | 15.1 | 4.2 | 151 | 3.6 | 47 | | | |
| | | 60° | | 114 | 71 | 82 | 33 | 19.5 | 13.8 | 5.7 | 141 | 2.5 | 75 | 77 | 110 | 70 |
| J. A. | Male, 49, Idiopathic postural hypotension | Flat | | | | 77 | 17 | 19.3 | 16.4 | 2.9 | 137 | 4.6 | | | | |
| | | 70° | | | | 63 | 30 | 19.6 | 13.1 | 6.5 | 138 | 2.1 | | 82 | 176 | 46 |
| C. H. | Male, 62, Idiopathic postural hypotension | Flat | 0 | 168 | 83 | 117 | 33 | 16.8 | 12.5 | 4.3 | 151 | 3.5 | 71 | | | |
| | | 60° | −5 | 85 | 56 | 66 | 37 | 17.7 | 9.0 | 8.6 | 154 | 1.8 | 94 | 56 | 112 | 51 |
| | Five days later | 60° | | 68 | 45 | 53 | 37 | 15.4 | 6.2 | 9.2 | 133 | 1.4 | 94 | 45 | 112 | 40 |
| | After 75 gm. albumin in 1,300 cc. saline | 60° | −5 | 143 | 79 | 108 | 45 | 13.0 | 7.7 | 5.3 | 127 | 2.4 | 69 | | | |
| A. B. | Male, 37, Diabetes, postural hypotension | Flat | | 132 | 85 | 103 | 37 | 19.7 | 14.6 | 5.1 | 142 | 2.8 | 91 | | | |
| | | 60° | | 72 | 46 | 57 | 38 | 19.9 | 9.7 | 10.2 | 149 | 1.5 | 104 | 55 | 103 | 54 |
| | After 75 gm. albumin in 1,300 cc. saline | Flat | | 167 | 94 | 123 | 23 | 15.3 | 12.7 | 2.6 | 140 | 5.4 | 99 | | | |
| | | 60° | | 140 | 92 | 108 | 43 | 15.9 | 10.5 | 5.4 | 137 | 2.5 | 92 | 88 | 187 | 46 |
| L. M. | Female, 36, Tabes, postural hypotension | Flat | +1 | 173 | 117 | 143 | 26 | 12.6 | 9.6 | 3.0 | 163 | 5.5 | 103 | | | |
| | | 60° | −3 | 74 | 45 | 57 | 22 | 13.2 | 6.9 | 6.3 | 167 | 2.6 | 118 | 40 | 85 | 47 |
| | After 75 gm. albumin in 1,300 cc. saline | Flat | +8 | 151 | 101 | 125 | 21 | 10.6 | 7.5 | 3.1 | 186 | 6.1 | 103 | | | |
| | | 60° | 0 | 189 | 127 | 154 | 33 | 11.2 | 7.5 | 3.7 | 173 | 4.7 | 144 | 123 | 157 | 77 |
| G. G. | Male, 30, Diabetes, irregular postural hypotension | Flat | −1 | 99 | 61 | 75 | 17 | 15.9 | 12.4 | 3.5 | 150 | 4.3 | 90 | | | |
| | | 60° | −7 | 85 | 60 | 68 | 34 | 16.9 | 7.8 | 9.1 | 177 | 2.0 | 123 | 91 | 200 | 47 |
| | After 75 gm. albumin in 1,300 cc. saline | Flat | +8 | 117 | 70 | 89 | 17 | 13.2 | 9.8 | 3.4 | 177† | 5.2 | 83 | | | |
| | | 60° | −2 | 108 | 64 | 80 | 24 | 12.9 | 7.7 | 5.2 | 179 | 3.4 | 94 | 91 | 141 | 65 |
| | Separate study | Flat | 0 | 115 | 67 | 88 | 19 | 17.2 | 13.1 | 4.1 | 192 | 4.7 | 95 | | | |
| | | 60° | −1 | 107 | 78 | 88 | 42 | 17.9 | 9.9 | 8.0 | 169 | 2.1 | 125 | 100 | 220 | 45 |
| T. I. | Male, 29, Tabes, irregular postural hypotension | Flat | +1 | 121 | 72 | 92 | 30 | 18.7 | 14.7 | 4.0 | 123 | 3.1 | 103 | | | |
| | | 60° | −2 | 118 | 80 | 95 | 37 | 18.9 | 14.1 | 4.8 | 123† | 2.6 | 123 | 103 | 123 | 84 |
| | After 75 gm. albumin in 1,300 cc. saline | Flat | +6 | 155 | 87 | 114 | 37 | 14.6 | 10.6 | 4.0 | 125 | 3.1 | 114 | | | |
| | | 60° | +5 | 151 | 89 | 115 | 34 | 14.8 | 11.4 | 3.4 | 115 | 3.4 | 109 | 101 | 92 | 110 |

* Samples collected approximately one minute apart.

† Calculation employs preceding oxygen consumption.

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[5] Observations on J. A. were made by Drs. Ebert and Borden of Minneapolis, Minnesota, who kindly made the data available for presentation in this paper.

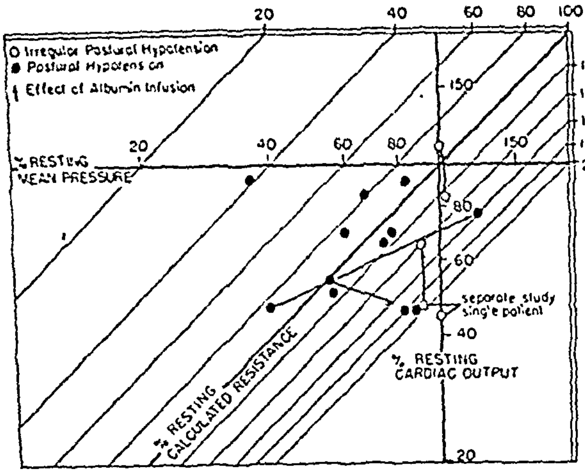

FIG. 1. EFFECT OF CHANGE FROM SUPINE TO UPRIGHT POSITION ON CARDIAC OUTPUT, MEAN ARTERIAL PRESSURE, AND CALCULATED RESISTANCE

The resting, supine state of each subject is represented by the origin. Each plotted point represents a determination in the tilted position. Changes in output, mean arterial pressure, and calculated resistance are expressed as percentage deviations from the resting levels, which are taken to be 100%. Arrows connecting two points indicate that an albumin infusion has been given to a particular subject. The point toward which the arrow is directed represents the effect of tilt after infusion, as referred to measurements in the supine position, also after albumin.

severe weakness and giddiness in the upright position, often had marked postural hypotension, but showed little or none during the study. These patients had no varicosities or other known anatomical abnormalities interfering with venous return. They are classified as having "irregular postural hypotension."

An over-all view of the results is provided by Figure 1. In this figure the resting, supine state of each subject is represented by the origin. Changes in cardiac output, mean arterial pressure, and calculated resistance during tilt are expressed for each subject by the location of a point indicating percentage deviation from the resting levels, which are taken to be 100%. Each plotted point represents a determination in the tilted position. Where two points are connected by an arrow, they both represent the same subject and indicate that an albumin infusion has been given. The point toward which the arrow is directed represents the condition during tilt after albumin has been given. These points, like the others, indicate percentage change from control measurements in the supine position. After the administration of albumin new control measurements were made in the supine position. The values obtained in the subsequent tilt were referred to these new control values.

In Figure 1, the average normal postural reaction would be represented by a region directly below the origin centering about the 75% level on the cardiac output axis. This corresponds to a maintenance of the resting mean arterial pressure, a fall of 25% in cardiac output, and an increase of about 33% in calculated resistance (6, 7).

The two subjects with "irregular postural hypotension" are represented in Figure 1 by open circles. One of these (T. I.) fell within the normal range, since he maintained his pressure and had only a small decline in output on tilt. The other (G. G.), who was studied twice, showed little or no pressure drop on tilt, but had a very large fall in cardiac output. Consequently, this subject had a great increase in calculated resistance during tilt. In terms of maintaining arterial pressure, both of these subjects at the time of study had an adequate vasoconstrictor response to a drop in cardiac output.

The other ten subjects are represented in the figure by solid circles. Eight of these showed little change in calculated resistance during tilt. In very broad terms, the greater the fall in cardiac output, the greater was the drop in arterial pressure, expressed as percentages. In three of these subjects (A. B., C. H., and L. M.) both cardiac output and arterial pressure fell below 60% of the resting levels.

The remaining two subjects show wide deviations from this broad picture. J. A. had a large drop in cardiac output but only an 18% decrease in pressure. There was a large rise in calculated resistance. His behavior is quite like that of G. G., who was classified as "irregular postural hypotension." The difference in classification, which is somewhat artificial, is based on the clear failure of the present subject to maintain his pressure on tilt at the time of study. The other subject, J. D., had the severest postural hypotension of the group. She often fainted in a chair. This patient had the greatest percentage fall in pressure on tilt, but there was little decline in output. There was a large decrease in calculated resistance. When this patient was tilted 30° head down there was a moderate fall in pressure despite an increased cardiac output. Again, there was a sizable drop in calculated resistance. The other patient (C. G.) who was tilted head down showed no change in output, pressure, or resistance.

In four subjects (C. G., C. C., J. A., and G. G.) the resting cardiac index was between 4.0 and 5.0, and in one (L. M.) it was 5.5. The largest cardiac index falls of the group occurred in three of these subjects during tilt. This suggests that anxiety may have played a part in some of the postural reactions.

Albumin was given to five subjects. In all cases the absolute values of cardiac output and arterial pressure were distinctly higher in the tilted position after albumin than in the tilted position before albumin. In four cases (A. B., L. M., G. G., and T. I.) data are available to describe the changes on tilt after albumin in terms of percentage deviation from the resting level. The results are represented in Figure 1 by the points toward which arrows are directed. The two patients with "irregular" postural hypotension (G. G. and T. I.) continued to maintain their blood pressures during tilt as they had before albumin. As compared with the pre-albumin state, the cardiac outputs were relatively higher and the increase in resistance relatively less. In the other two patients, who had well-marked postural hypotension, the most striking change after albumin was the improvement in maintenance of arterial pressure during tilt. One subject showed a much smaller percentage fall in output than before; the other had a slightly greater percentage fall. Before albumin, neither subject had much change in calculated resistance during tilt. After albumin both cases showed a well-marked increase in calculated resistance during tilt. The increase in calculated resistance was unexpected. It suggests an increase in arteriolar reactivity after albumin, but the expression for resistance is not sufficiently exact to make this secure.

## DISCUSSION

In the over-all view, significant postural falls in blood pressure were more consistently associated with failure of normal arteriolar constriction (as indicated by lack of increase in calculated resistance) than with abnormal decline in cardiac output. With a single exception, the subjects with significant postural hypotension showed on tilting a very minor increase in peripheral resistance or an actual fall. This is in general agreement with the findings of Stead and Ebert (8), who concluded on other grounds that failure of arteriolar constriction in response to a fall in blood pressure is fundamental to the production of postural hypotension. A similar conclusion was reached by Ellis and Haynes (9), who measured the cardiac output of two subjects by the acetylene method and found no more than a normal decrease in the tilted position.

One subject (J. D.), who had the most severe postural hypotension of the group, showed a considerable fall in calculated resistance on being tilted in either direction. The mechanism of this fall is not clear. It may result from passive dilatation of arterioles below heart level under increased hydrostatic pressure, thus providing a wide outflow bed. This possibility was suggested by Ellis and Haynes.

The postural fall in cardiac output was variable. From inspection of Figure 1 it is apparent that significant postural hypotension can occur without a greater than normal fall in cardiac output, and that a very large postural fall in cardiac output can occur without a significant drop in blood pressure. However, large pressure drops are often associated with large drops in output. The increase in actual output of tilted subjects after albumin suggests that these large output falls resulted from an inadequate venous return to the heart. The most apparent cause for inadequate return is abnormal dilatation of the venous system under pressure. Filling this system with a larger blood volume would improve venous return to the heart. Normally, the venous system resists passive dilatation under hydrostatic pressure in the upright position, and venous return is well maintained.

Active venous constriction is a familiar response to circulatory inadequacy, as in shock and congestive heart failure. It can occur rapidly in acute pericardial tamponade (10). It is quite possible that an increase in venous tone is an important, normal response to assuming the upright position. The present results suggest that such a response would assist in maintaining the cardiac output, just as arteriolar constriction maintains the blood pressure.

## SUMMARY AND CONCLUSIONS

1. Observations have been made on postural reactions of cardiac output and blood pressure in 12 subjects with postural hypotension.

2. Well-marked and even extreme postural hypotension can occur on the basis of failure of arteriolar constriction with no more than a normal postural decrease in cardiac output. Slight to moderate falls in mean arterial pressure have been observed in subjects with very large postural falls in cardiac output and large, though not completely compensatory, increases in calculated resistance. However, large postural falls in arterial pressure were often associated with large falls in cardiac output. In these cases the calculated resistance showed little change on tilt.

3. Large infusions of human serum albumin were given in five cases. In each case there was an increase in cardiac output in the tilted position as compared with the cardiac output in the tilted position before albumin infusion. Arterial pressure was well maintained during tilt. In two cases the response of calculated resistance to posture was greatly increased by the infusion. In two cases, the response was decreased.

4. The data suggest that postural hypotension is primarily dependent upon inadequate arteriolar tone in the upright position. The degree of postural hypotension is often intensified by a large postural fall in cardiac output. It is suggested that this may result from an associated deficiency in venous tone.

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# QUANTITATIVE DETERMINATION OF THE VISCERAL PAIN THRESHOLD IN MAN

## CHARACTERISTICS OF VISCERAL PAIN, EFFECT OF INFLAMMATION AND ANALGESICS ON THE THRESHOLD, AND RELATIONSHIP OF ANALGESIA TO VISCERAL SPASM

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(Submitted for publication November 15, 1950; accepted, February 16, 1951)

The development of synthetic analgesics and derivatives of morphine has focused much attention on analgesic testing methods (1). Goetzl, Burrill and Ivy in 1943 abstracted from the literature 75 different methods of quantitative measurement of the pain threshold (2). Pain producing stimuli were divided into mechanical, thermal, chemical and electrical, and all have been used on experimental animals as well as human volunteers. These various stimuli have been applied only to integumental structures, such as skin, lips, teeth and cornea (3, 4). It is generally recognized that visceral pain is a different sensation, elicited only by a special set of stimuli and mediated to the central nervous system through different pathways (5). Little work has been done in the field of visceral algesimetry. Our present evaluation of the effectiveness of various analgesic drugs is based partly on clinical impressions and partly on the assumption that an analysis of response to superficial pain provides an evaluation which also holds true for "deep" or visceral pain (6, 7).

Many of the notorious difficulties of integumental pain threshold determination apply equally to visceral pain. Added to these is the obvious difficulty of inaccessibility of visceral organs. Furthermore, viscera do not respond to the more easily quantitated thermal and electrical stimuli. They respond to only one special mechanical stimulus, that of stretch. From available data it is apparent that visceral pain responses, when obtained, vary a great deal more in intensity, quality and localization than pain responses from integumental structures. In spite of this, a number of valuable investigations has been made concerning localization of pain produced by distention of various portions of the gastro-intestinal tract with balloons.

Quantitative studies of deep analgesia were conducted by Wolff, Hardy and Goodell (6) and Harrison and Bigelow (7) using the method of Lewis, Pickering and Rothschild (8) by which pain is produced by the rhythmic contraction of ischemic muscle of the forearm. From these studies it was concluded that the effects of analgesics on deep pain are similar to those on superficial pain.

The threshold raising effects of narcotics on visceral pain were noted in trained dogs by Yonkman (9). Wolff, Hardy, and Goodell have studied qualitative effects of opiates on visceral pain in man by distending balloons in the duodenum for 40 minutes (6).

The gall bladder and biliary tract, though ordinarily inaccessible, become a fertile region for visceral pain studies after surgical drainage and intubation of any portion of this system. Localization of referred pain from the gall bladder has been studied by distending this organ during cholecystectomy (10, 11), by distending the gall bladder and common bile duct during abdominal operations under local anesthesia or after general anesthesia had worn off (12), and by faradic stimulation of the common bile duct through an electrode incorporated in a common duct catheter (13). Layne and Bergh studied the duration of biliary pain as related to biliary spasm by increasing the intrabiliary pressure with saline solution through a common duct tube (14), and White using the same technique investigated the effect of splanchnicectomy on biliary pain (15).

McGowan and associates observed intrabiliary pain and pressures in patients after choledochostomy by connecting the T-tube in the common bile duct to a manometric system filled with sterile saline solution (16, 17). From these studies they evolved criteria for removal of the T-tube. One of these criteria was that the "perfusion pain pressure" produced by external elevation of the hydrostatic pressure within the biliary system should be over 500 mm. of water. They felt that pain sensations at lower hydrostatic pressures indicated residual cholangitis. McGowan and the author have utilized these post-operative studies for investigation of a variety of drugs thought to have spasmolytic action on the biliary tract (18-20). Opiates and synthetic analgesics were frequently given during the course of these studies for the production of artificial biliary and duodenal spasm. These drugs were found to raise the "perfusion pain pressure" and the elevation was roughly proportional to the potency and the dosage of the drug. This suggested the use of the T-tube as a pathway to visceral algesimetry.

## METHOD

Forty patients volunteered for this study following surgical exploration and intubation of the common bile duct. Of these, 38 also had the gall bladder removed; one patient merely had an exploration and intubation of the common duct and one patient had an exploration of the common duct with a long arm of the T-tube placed through the sphincter of Oddi into the duodenum (Table I).

The original method of McGowan, Butsch and Walters, proposed for intrabiliary pressure recording was employed (16). Two weeks or more after operation, patients were placed in the supine position and the T-tube emerging from the abdomen was connected to a three way manometric system. One limb was connected to an infusion bottle containing sterile saline solution. This permitted filling of the entire system and the biliary ducts with solution. The second limb was connected to a short manometer with a small glass bubble as float arranged to record biliary pressures on a slowly revolving smoked drum. The third limb was connected to a long manometer graduated in mm. which permitted reading of hydrostatic pressures when the infusion bottle was connected to the system and elevated. At times this third water manometer was replaced by a mercury manometer arranged to record pressures on the smoked drum. For all pressure recordings the patients' xyphoid process was chosen arbitrarily as the reference zero point.

The pain threshold was obtained in the following manner: The resting-pressure manometer, being too short for recording of pressures over 250 mm. of water was momentarily cut off from the circuit and the infusion bottle was introduced to the system. The bottle was then slowly raised behind a screen to prevent patients from observing its height. Patients were asked to call out when the first painful sensation was noted. The intrabiliary pressure was recorded at this level by direct reading or by the mercury manometer on the smoked drum. The resting pressure manometer was then substituted for the infusion bottle into the circuit. After each threshold determination, patients were asked to describe the quality, intensity and location of the sensation.

*Not all patients tested were suitable subjects for repeated pain threshold recordings.* Of the 40 patients who volunteered only 32 could be called upon for further studies. Unsuitable subjects could be divided into three groups. Three patients experienced no sensation of any kind up to pressures of 1,000 mm. of water. Higher pressures were never used. Sufficiently high pressures to cause pain could never be obtained in one patient where the long arm of the T-tube was in the duodenum since infusion fluid flowed into the intestine too rapidly for pressure elevation to develop. In four patients, colicky abdominal pain was easily induced by increased pressures but unlike most patients the pain did not subside together with the fall in intrabiliary pressure. In three patients, colicky pain persisted for five to 10 minutes in spite of normal intrabiliary pressures and medication was required to stop the persistent pain of the fourth patient. These patients were unsuitable for repeated pain level recordings and incidentally only these four patients were unwilling to continue with further experiments.

Resting biliary pressures were recorded for 30 minutes. During this time control pain thresholds were recorded at least three times. These usually checked within 30 mm. of water (Table I). Rapid elevation of the infusion bottle tended to result in slightly higher threshold readings. In later experiments, the speed of pressure elevation was standardized to 30 mm. per second so that even the highest threshold elevations could be obtained in 30 seconds.

After 30 minutes, the analgesic drug to be tested was given by the parenteral route. Pain threshold recordings were made 15 and 30 minutes and one, two and three hours after injection. Occasionally in cooperative patients, thresholds were determined at more frequent intervals. On other occasions experiments were prolonged to four and five hours.

*Treatment of data:* Both the spontaneous or resting intrabiliary pressure and the pressure sufficient to cause pain were measured in mm. of water with the patients' xyphoid process as the zero reference point. Resting pressure elevations due to the spasmogenic effect of drugs were expressed as elevation in mm. of water since initial resting pressures in patients without biliary sphincter obstruction varied little between 10 and 50 mm. of water (16, 18, 19). Pain threshold elevations due to the analgesic effects of the drugs were measured both in mm. of water and as per cent elevation. The latter form was more useful since initial control pressures necessary to cause pain varied from 90 to 800 mm. of water.

Another value, representing the total analgesic effect of the drug, was obtained by measuring the area under the time-action curve (6). This reflected both the threshold raising activity and the duration of action. The unit for this measure of activity was "per cent-hours."

Peak resting pressures were obtained from the highest point of the kymograph tracings. Peak pain threshold levels were the highest thresholds measured during a three to four hour period of study. For calculation of the average result of several patients the arithmetic mean was used.

## RESULTS

*Character and location of pain:* Hydrostatic distention of the biliary ducts with pressures up to 800 mm. of water caused some pain sensation in 36 of 40 patients (Table I). The quality and intensity of the pain was described as sharp, stabbing or colicky by 17 patients and was called dull or just an "ache" by 17 other patients. Only two patients noted discomfort which they could not actually call pain with no further increase of this unpleasant sensation up to pressures of 1,000 mm. of water.

The sensation was well localized in 27 patients, usually in the right upper quadrant of the abdomen (23 patients) and occasionally in the epigastric region (4 patients). Five subjects reported pin point localization at a point just below the costal margin in the midclavicular line. The sensation had a more vague distribution in nine patients; it was referred to the entire abdomen by three and to the upper part of the abdomen by six. Some radiation to the back was noted by nine patients; this radiation was never well localized and usually referred to the right upper part of the back. Pain chiefly on the left side over the precordium or radiating down the arm was never reported.

There appeared to be no relationship between the quality and intensity of the preoperative pain and the type of pain sensation reported with these experiments. There was a definite relationship

TABLE I

*Location and character of induced biliary pain and its relation to preoperative pain*

| Case No. | Age | Sex | Duration of intubation | No. of experiments | Postoperative pain threshold | Final pain threshold | Location of preoperative pain | Experimental threshold location | Pain type |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 29 | F | 3 months | 5 | 440–450 | 620–630 | Epigastrium and back | Epigastric and infrascap. | Sharp |
| 2 | 58 | F | 50 days | 6 | 350 | 500 | Right upper quadrant | Right upper quadrant | Ache |
| 3 | 71 | M | 3 months | 6 | 700 | 700–720 | Right upper quadrant | Right upper quadrant | Stabbing |
| 4 | 55 | F | 2 months | 7 | 620–650 | 700–710 | None, nausea, vomiting | Right upper quadrant | Dull |
| 5 | 50 | F | 40 days | 7 | 700 | 700 | Right upper quadrant | Right upper quadrant | Dull |
| 6 | 23 | F | 49 days | 7 | 90–100 | 260–265 | Right upper quadrant | Right upper quadrant | Discomf. |
| 7 | 62 | M | 26 days | 4 | 400–430 | 700–720 | Generalized abdominal | RUQ and epigastric | Dull |
| 8 | 40 | F | 29 days | 9 | 320–330 | 600 | None, vomiting and jaundice | Right subcostal | Sharp |
| 9 | 32 | F | 43 days | 4 | 380 | 510 | Entire abdomen | RUQ and infrascapular | Sharp |
| 10 | 55 | M | 17 days | 4 | 310–330 | 400–430 | Painless jaundice | Upper abdomen | Dull |
| 11 | 66 | F | 22 days | 4 | 450–460 | 470 | Upper abd., rad. to back | RUQ radiating to back | Sharp |
| 12 | 67 | F | 22 days | 1 | — | — | Right upper abdomen | Lasting pain spasm | |
| 13 | 66 | M | 63 days | 7 | 260–270 | 290–300 | Upper abdomen | Upper abdomen | Dull |
| 14 | 67 | F | 6 months | 3 | 280–300 | 400 | Sharp, rt. upper abdomen | Right upper quadrant | Stabbing |
| 15 | 53 | F | 18 days | 1 | — | 500–520 | Rt. upper abd., rad. to back | RUQ radiating to back | Dull |
| 16 | 37 | M | 68 days | 4 | 160–180 | 470 | Post-gastrectomy jaundice | Right subcostal | Sharp |
| 17 | 56 | M | 11 months | 1 | — | — | Operative ligation of C.D. | T-tube in duodenum | |
| 18 | 44 | M | 14 days | 1 | 1,000+ | — | Generalized abdominal | No pain to 1,000 mm. | |
| 19 | 57 | F | 43 days | 8 | 400–410 | 490–500 | Colicky rt. flank pain | Right upper quadrant | Stabbing |
| 20 | 64 | F | 40 days | 2 | 220–230 | 240–250 | Right upper quadrant | Upper abdomen | Discomf. |
| 21 | 38 | M | 70 days | 1 | — | 700 | Rt. upper abd., rad. to back | Right upper quadrant | Sharp |
| 22 | 34 | F | 7 months | 7 | 600–620 | 700 | Jaundice, clay col. stools | Epigastric | Dull |
| 23 | 53 | F | 52 days | 4 | 590–600 | 620–630 | Epigastric | Epigastric | Dull |
| 24 | 41 | F | 4 months | 1 | 1,000+ | — | Epigastric | No pain to 1,000 mm. | |
| 25 | 39 | M | 5 months | 7 | 200–210 | 200–220 | Right upper quadrant | Epigastric | Sharp |
| 26 | 53 | F | 22 days | 2 | 600–610 | 600–610 | Gen. abd., rad. to back | Upper abd. and back | Dull |
| 27 | 57 | F | 42 days | 1 | — | — | Sharp, rt. upper abdomen | Lasting pain spasm | |
| 28 | 38 | F | 24 days | 3 | 700–710 | 710 | Epigastric | RUQ and epigastric | Dull |
| 29 | 59 | F | 55 days | 1 | — | — | Substernal distress | Lasting pain spasms | |
| 30 | 54 | M | 14 days | 2 | 800 | 800 | Midabdominal | Upper abdomen | Dull |
| 31 | 66 | F | 25 days | 7 | 280–290 | 300–320 | Epigastric | RUQ and epigastric | Sharp |
| 32 | 29 | F | 62 days | 10 | 140–150 | 300 | Right upper quadrant | Rt. upper abd. and back | Sharp |
| 33 | 52 | F | 18 days | 3 | 600 | 660–670 | Epigastric | Rt. subcostal and epig. | Dull |
| 34 | 40 | M | 21 days | 3 | 700 | 700 | Lower abdomen | Right upper quadrant | Sharp |
| 35 | 43 | F | 14 days | 1 | — | — | Epigastric burning | Lasting pain spasms | |
| 36 | 40 | F | 26 days | 7 | 550 | 560–670 | Painless, jaundice, nausea | Generalized abdominal | Dull |
| 37 | 53 | F | 23 days | 7 | 320–330 | 400 | Upper abdomen | Right upper quadrant | Dull |
| 38 | 30 | F | 17 days | 7 | 300 | 310–320 | Rt. upper abd., rad. to back | Rt. upper abd. and back | Sharp |
| 39 | 41 | F | 14 days | 1 | 1,000+ | — | Generalized abdominal | No pain to 1,000 mm. | |
| 40 | 36 | F | ? | 1 | 290–300 | — | Nausea, vomiting | Right subcostal | Dull |

between the preoperative and postoperative localization of the pain: of six patients who named pain radiating to the back as their chief complaint on entry to the hospital, five later reported back pain as part of the sensation due to distention of the biliary ducts. Chapman and associates have previously reported on this relationship (21). Repeated distention of the biliary system in the same patients resulted in identical localization, intensity and quality of pain both during the course of the same study and on different days. The intensity, quality and localization of the pain was not altered by the rapidity with which the pressure was elevated.

*The pain threshold:* A great deal of variation was found in the level of the pain threshold from one patient to another. The pain threshold of all patients 14 days after introduction of the T-tube varied from 90 to 800 mm. of water and at the time of tube removal from 200 to 800 mm. of water (Table I). There was less variation of the threshold in the same patients tested at various times. In 15 subjects, mostly those with high initial threshold, the pain level varied no more than 30 mm. during the entire time of intubation, as long as five months in one instance. In the remaining 21 patients, the thresholds slowly increased during the course of observation. A decrease of the threshold was never noted. A slowly increasing threshold over the course of weeks was interpreted as a sign of subsiding cholangitis (16). Sarkisian and McGowan have found a good relationship between the level of the pain threshold and the bacterial count of the bile (22). Similarly inflammation was found to lower the pain threshold during tests of skin pain (23).

In spite of these variations, the biliary pain threshold was found useful as a measure of the effect of analgesic and narcotic substances because variations of threshold in the same patient during the course of a single study were very small (Table I). In 10 subjects repeated threshold determinations on the same day varied by less than 5 mm. of water while in the remaining 24 patients the variation was less than 30 mm. of water, usually no more than 10 mm. In addition, in 15 subjects the threshold varied very little from one day to another often for many weeks and even months (Table I). These patients were well suited for comparative study of the action of several drugs.

TABLE II

*Individual threshold determinations of six patients during 12 hours*

| Time | | Patient No. | | | | | |
|---|---|---|---|---|---|---|---|
| Hours | Min. | 28 | 31 | 32 | 36 | 37 | 38 |
| 0 | 0 | 700 | 280 | 140 | 560 | 340 | 305 |
| 0 | 10 | 700 | 280 | 165 | 560 | 360 | 310 |
| 0 | 20 | 705 | 290 | 145 | 565 | 370 | 310 |
| 0 | 30 | 710 | 290 | 140 | 565 | 375 | 310 |
| 1 | 0 | 705 | 290 | 140 | 560 | 370 | 310 |
| 1 | 30 | 705 | 290 | 140 | 570 | 345 | 320 |
| 2 | 0 | 710 | 295 | 145 | 570 | 340 | 320 |
| 2 | 30 | 710 | 290 | 140 | 570 | 350 | 320 |
| 3 | 0 | 710 | 295 | 145 | 570 | 350 | 320 |
| ..... | ..... | | | | | | |
| 6 | 0 | 695 | 300 | 150 | 555 | 340 | 310 |
| 6 | 15 | 695 | 295 | 150 | 570 | 350 | 310 |
| ..... | ..... | | | | | | |
| 8 | 0 | 710 | 290 | 150 | 560 | 350 | 320 |
| 8 | 15 | 710 | 290 | 145 | 570 | 360 | 320 |
| ..... | ..... | | | | | | |
| 11 | 0 | 710 | 310 | 155 | 560 | 375 | 315 |
| 11 | 15 | 705 | 300 | 150 | 565 | 370 | 320 |
| 11 | 30 | 705 | 300 | 150 | 570 | 370 | 320 |
| 12 | 0 | 710 | 295 | 145 | 570 | 370 | 325 |
| Mean, mm. of water | | 706 | 293 | 147 | 565 | 358 | 316 |
| Standard deviation, mm. | | ±5.1 | ±7.1 | ±5.1 | ±5.0 | ±12.6 | ±5.6 |
| Coefficient of variation, per cent | | 0.67 | 2.43 | 3.82 | 0.88 | 3.52 | 1.77 |

Near the completion of this study it appeared desirable to obtain data for statistical expression of threshold variations in individual patients. Six patients were studied by repeated threshold determinations over the course of 12 hours (Table II). At intervals these patients returned to the ward for meals and visiting hours and studies were then continued. In all, 17 control readings were obtained. The mean pain thresholds varied from 146.7 to 705.6 mm. of water in these six patients. The smallest standard deviation was ± 5.1 mm. for a mean pressure of 705.6 mm. and the largest standard deviation encountered was ± 12.6 mm. for a mean pressure of 357.9 mm. The smallest coefficient of variation was 0.67 per cent and the largest was 3.82 per cent (Table II). These figures from six selected patients compared favorably with data obtained by the Hardy-Wolff-Goodell technique of individual patients studied from time to time. Uniformity of pain threshold from individual to individual obtained by thermal radiation of the skin could not be duplicated with visceral pain threshold determinations.

*The end point:* The threshold end point was usually definite particularly in patients who experienced sharp or stabbing pain. A little more care was necessary to determine the exact end point in those patients who experienced merely a dull ache. In the 32 patients found suitable for visceral pain threshold studies the pain sensation

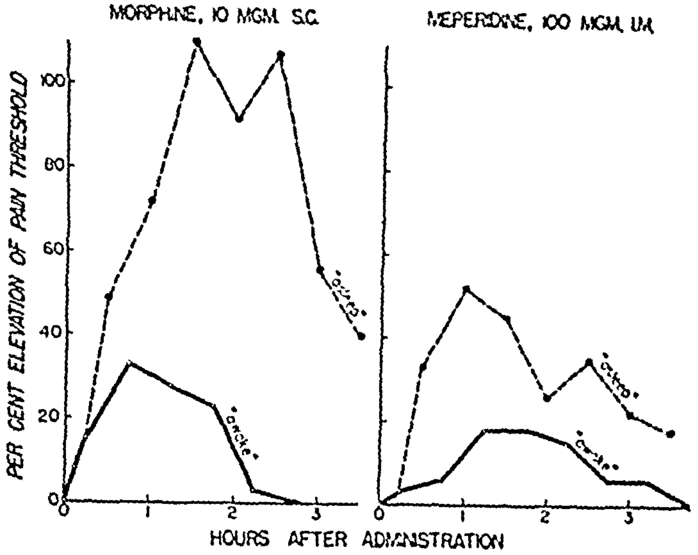

Fig. 1. Perception of and Reaction to Visceral Pain

For "awake" threshold determinations patients were aroused before the test and asked to signify the first unpleasant sensation due to elevation of the intrabiliary pressure. For the "asleep" curves the pressure was elevated without warning to the inattentive, drowsy or sleeping patients.

subsided almost instantly as the pressure was reduced. In no case was there pain for more than 15 seconds with each threshold determination.

Experiences with pain produced by inflation of balloons in the esophagus, duodenum, colon and ureter suggest that the biliary tree is the most suitable hollow viscus for determination of pain thresholds. Precise hydrostatic measurements of the pain threshold in these other organs were not possible in our experience because these organs responded with peristaltic waves to even slight pressure elevations in the balloon. These contractions were reflected in the pressure readings and steady high pressures within the balloon lumen could therefore not be maintained. Relative to the size of its lumen the common bile duct has by far the thinnest muscular wall of any of the above named organs. Here peristaltic movements are usually not observed during recording of resting pressures. Even with the more sensitive and rapidly responding microphone-condenser electromanometer the only pressure changes recorded were due to changes in abdominal pressure with cardiac systole, respirations and voluntary contractions of the abdominal musculature.

During routine experiments patients were invariably warned of the approaching threshold determination to assure their alertness and then the saline infusion bottle was raised behind the screen. Experiments were also planned during which the patient was notified of the pressure elevation about to occur at the end of 15 and 30 minutes and one, two and three hours and at irregular intervals between these periods the infusion bottle was raised without the patient's knowledge. Figure 1 illustrates two such time action curves of two different patients. Prior to administration of the drug there was no difference between the "awake" and "asleep" thresholds. Fifteen minutes after injection there was a definite elevation of the threshold both after 10 mgm. of morphine and 100 mgm. of meperidine. The two thresholds were still the same. One hour after morphine, there was an "awake" threshold elevation of 30 per cent while the "asleep" threshold elevation was 72 per cent.

TABLE III

*Visceral analgesic activity of narcotics and acetylsalicylic acid*

| Drug | No. of patients | No. of studies | Mean peak threshold elevation, mm. water | Mean peak threshold elevation, per cent | Analgesic activity, "per cent-hours" |
|---|---|---|---|---|---|
| Morphine sulfate, 10 mgm., s.c. | 30 | 40 | 233 | 56.4 | 140 |
| Codeine sulfate, 60 mgm., s.c. | 22 | 27 | 53 | 11.9 | 40 |
| Dihydromorphinone hydrochloride, 3 mgm., s.c. | 11 | 11 | 162 | 41.9 | 112 |
| Methyldihydromorphinone hydrochloride, 7.5 mgm., s.c. | 7 | 7 | 162 | 46.4 | 98 |
| Methadone, 7.5 mgm., s.c. | 10 | 10 | 153 | 33.2 | 168 |
| Meperidine hydrochloride, 100 mgm., c.m. | 32 | 50 | 102 | 22.9 | 70 |
| Acetylsalicylic acid, 0.6–1.2 Gm., oral. | 12 | 13 | 0 | 0 | 0 |

The peak threshold elevations for these two types of determinations were 33 and 110 per cent, respectively, after morphine and 18 and 53 per cent, respectively, after meperidine. Three hours after injection of morphine the "awake" threshold was no longer elevated while the "asleep" threshold was still 40 per cent above the control level.

The first type of threshold, called "awake" was the true perception threshold since the end point was given by alerted patients' first recognition of a painful sensation. The second or "asleep" value was obtained after drowsy, inattentive or even asleep patients were sufficiently stimulated by the pain to make their complaint known. This was therefore not a true threshold but a measure of the analgesic action together with the physiologic and psychologic response to the hypnotic, euphoria producing and other effects of the narcotic. It is apparent that these other effects contribute greatly to the clinical effectiveness of narcotics on visceral pain. Furthermore hypnosis, euphoria and inattentiveness were in evidence for a much longer period of time than the analgesic effect.

*Effect of analgesic drugs on the visceral pain threshold:* Satisfactory time action curves were obtained from 32 patients on 158 occasions. No ill effects were encountered during or after these studies. Two patients experienced chills about three hours after threshold studies and cholangiography. The temperature, elevated at that time, was found normal three hours later and remained normal thereafter.

The analgesic activity of drugs was compared in two groups of patients. In the first all the patients studied were included (Table III). In the second only seven patients were included whose threshold response was measured for all seven drugs of this series (Figure 2). A true comparison of the effectiveness of the various analgesics could best be made in the latter group since individual initial pain thresholds varied widely and the analgesic effect was dependent to some extent on the magnitude of the initial threshold. These seven patients were among the last ones studied since some of the newer synthetic analgesics had not been available previously; they were patients who showed a good pain threshold end point and whose control thresholds varied little from day to day; lastly they were chosen because the T-tube drainage was continued for a sufficient period of time to allow for evaluation of all drugs. Studies were prolonged to four and one-half hours in this group.

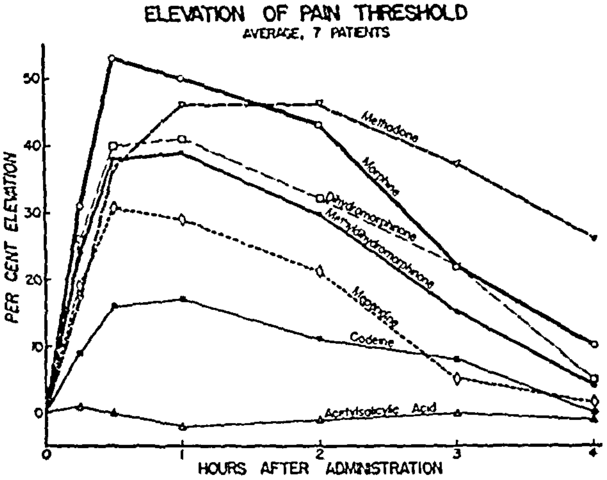

FIG. 2. MEAN TIME-ACTION CURVES IN PER CENT ELEVATION OF PAIN THRESHOLD FOR SEVEN PATIENTS AFTER VARIOUS NARCOTICS AND ACETYLSALICYLIC ACID

*Morphine sulfate:* Ten mgm. of this agent were given subcutaneously to 30 patients on 40 occasions (Table III). A marked elevation of the visceral pain threshold was observed every time. The onset of action was rapid. The threshold was elevated after 15 minutes in every instance and occasional studies seven minutes after injection showed increase of pressure tolerance at that time. The peak action was observed 15 minutes after injection in 10 per cent of the time-action curves, after 30 minutes in 70 per cent and after one hour in 20 per cent. Some action was still discernible after three hours in all but one patient. The mean peak elevation from the control level was 233 mm. of water or 56.4 per cent and the total analgesic effect was 140 per cent-hours (Table III).

The pain thresholds after morphine of the seven patients in the second group are shown in Table IV. Definite threshold elevation was still present in all patients at the end of four hours. The mean peak elevation of 53 per cent was found 30 minutes after injection.

The mean time-action curves for all drugs for the seven patients of the second group are shown in Figure 2. With the dosage schedule used, morphine caused the highest peak effect and its duration of action was surpassed only by methadone.

*Codeine sulfate:* This drug was given subcutaneously in 60 mgm. doses to 22 patients on 27 occasions (Table III). A threshold elevation resulted in all but one patient. The onset of action was slower than after other drugs. Only two-thirds of the time-action curves indicated activity 15 minutes after injection. The height of the effect was reached 30 minutes after administration on 10 occasions and one hour after injection on 16 occasions. The duration of action was briefer than that of morphine. Only 72 per cent of the time-action curves showed threshold elevation after three hours. However, all elevations after codeine were so small that the method may well not have been sufficiently delicate to demonstrate slight effects at both ends of the time-action curves. The mean peak elevation for all patients was 53 mm. of water or 11.9 per cent. The total analgesic action of 40 per cent-hours was the smallest encountered.

Comparison of the responses of the seven patients treated with all drugs showed relatively greater variations than those of any other group (Table IV). The mean time-action curve (Figure 2) indicated that codeine caused the smallest threshold elevation of any drug studied. There was no discernible effect at the end of four hours.

*Dihydromorphinone hydrochloride ("Dilaudid")*: Three mgm. of this drug were given subcutaneously to 11 patients (Table III). The first response, like that after morphine was noted after seven minutes. The peak response was obtained 30 minutes after injection in four patients and one hour after treatment in the remainder. In the

TABLE IV

*Visceral pain threshold elevation in mm. of water of seven individual patients after various narcotics*

| Patient No. | Time after administration | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Minutes* | | | *Hours* | | | | *Minutes* | | | *Hours* | | | |
| | 0 | 15 | 30 | 1 | 2 | 3 | 4 | 0 | 15 | 30 | 1 | 2 | 3 | 4 |
| | Morphine sulfate, 10 mgm. | | | | | | | Methyldihydromorphinone HCl, 7.5 mgm. | | | | | | |
| 22 | 620 | 640 | 680 | 680 | 670 | 630 | 630 | 200 | 320 | 390 | 420 | 350 | 320 | 250 |
| 25 | 190 | 360 | 410 | 480 | 390 | 340 | 250 | 300 | 380 | 450 | 450 | 410 | 400 | 330 |
| 31 | 300 | 400 | 480 | 500 | 460 | 400 | 360 | 300 | 370 | 350 | 350 | 330 | 330 | 330 |
| 32 | 140 | 300 | 380 | 370 | 340 | 280 | 230 | 560 | 610 | 610 | 610 | 600 | 580 | 550 |
| 36 | 560 | 620 | 620 | 510 | 600 | 570 | 570 | 320 | 460 | 520 | 530 | 480 | 400 | 330 |
| 37 | 330 | 400 | 540 | 560 | 540 | 400 | 370 | 310 | 500 | 580 | 460 | 400 | 390 | 370 |
| 38 | 300 | 480 | 630 | 550 | 500 | 360 | 330 | 600 | 570 | 670 | 780 | 770 | 560 | 540 |
| Mean per cent elevation | 0 | 31 | 53 | 50 | 43 | 22 | 10 | 0 | 24 | 38 | 39 | 29 | 15 | 4 |
| | Codeine sulfate, 60 mgm. | | | | | | | Methadone, 7.5 mgm. | | | | | | |
| 22 | 600 | 620 | 640 | 650 | 620 | 620 | 620 | 700 | 730 | 750 | 770 | 775 | 780 | 740 |
| 25 | 210 | 260 | 290 | 300 | 240 | 220 | 200 | 210 | 260 | 320 | 380 | 420 | 390 | 360 |
| 31 | 320 | 360 | 400 | 400 | 380 | 360 | 330 | 290 | 320 | 400 | 480 | 460 | 450 | 390 |
| 32 | 160 | 200 | 220 | 240 | 190 | 180 | 150 | 150 | 170 | 320 | 360 | 300 | 210 | 190 |
| 36 | 550 | 550 | 590 | 590 | 580 | 580 | 570 | 550 | 580 | 590 | 610 | 610 | 600 | 570 |
| 37 | 360 | 430 | 360 | 360 | 370 | 360 | 350 | 310 | 400 | 480 | 500 | 520 | 450 | 430 |
| 38 | 300 | 300 | 410 | 390 | 390 | 370 | 340 | 300 | 480 | 580 | 570 | 570 | 550 | 510 |
| Mean per cent elevation | 0 | 9 | 16 | 17 | 11 | 8 | 2 | 0 | 17 | 37 | 46 | 46 | 37 | 27 |
| | Dihydromorphinone HCl, 3 mgm. | | | | | | | Meperidine HCl, 100 mgm. | | | | | | |
| 22 | 600 | 620 | 660 | 670 | 630 | 610 | 610 | 600 | 660 | 650 | 650 | 600 | 590 | 600 |
| 25 | 210 | 340 | 380 | 400 | 320 | 230 | 200 | 200 | 340 | 350 | 340 | 300 | 210 | 210 |
| 31 | 300 | 430 | 470 | 480 | 460 | 420 | 350 | 340 | 460 | 490 | 470 | 440 | 400 | 375 |
| 32 | 160 | 280 | 350 | 360 | 340 | 280 | 260 | 210 | 300 | 350 | 340 | 310 | 245 | 225 |
| 36 | 550 | 575 | 600 | 600 | 560 | 560 | 560 | 550 | 670 | 580 | 590 | 580 | 540 | 540 |
| 37 | 370 | 420 | 520 | 520 | 520 | 440 | 430 | 375 | 480 | 480 | 470 | 450 | 400 | 380 |
| 38 | 300 | 460 | 500 | 480 | 450 | 500 | 470 | 300 | 400 | 480 | 470 | 430 | 320 | 320 |
| Mean per cent elevation | 0 | 26 | 40 | 41 | 32 | 22 | 6 | 0 | 29 | 31 | 29 | 21 | 5 | 2 |

dosages used, dihydromorphinone was superior to methyldihydromorphinone in both peak per cent elevation and total analgesic action. Inspection of individual time-action responses (Table IV) and of their mean curve (Figure 2) revealed fairly uniform action in different patients. The peak of the curve was lower than that of morphine but at three hours their action was identical.

*Methyldihydromorphinone hydrochloride*[1] (*"Metopon"*): This morphine derivative was given in 7.5 mgm. doses to the seven patients of Table IV. Responses differed little from those obtained with dihydromorphinone. The onset of action was fairly rapid. In one patient the peak was reached in 15 minutes. Of the six others, three showed the maximal response after 30 minutes and three after one hour (Table IV). The shape of the time-action curve was almost identical to that of dihydromorphinone.

*Methadone:* Ten patients were treated with 7.5 mgm. of this agent (Table III). Although the first analgesic action was noted as soon after injection as after other drugs, the peak activity was definitely delayed. Only one patient showed maximal action after 30 minutes, four showed the peak after one hour, four after two hours and one after three hours. Since methadone was the only drug tested which showed useful activity at the end of four hours threshold determinations were continued beyond four hours in three patients. Useful activity of methadone at five hours was noted in all three and a mean elevation of 8 per cent was still found after six hours. The peak elevation for all ten patients was 153 mm. of water—less than that after morphine and its derivatives. Comparison of the individual (Table IV) and average time-action curves (Figure 2) of seven patients showed the peak action of methadone to be inferior only to morphine. At the end of two hours, methadone was superior to morphine and at three and four hours the activity of this drug was about twice that of morphine and its derivatives.

*Meperidine hydrochloride* (*"Demerol"*): One-hundred mgm. of this drug were given intramuscularly to all 32 patients on 50 occasions (Table III). It was the most rapidly acting drug of this series. Maximal action was noted after 15 minutes in one-half of the time-action curves while the remainder showed peaks 30 minutes after injection. Meperidine was also the shortest acting drug. Ten curves showed no activity after two hours and one-half of the tests showed no threshold elevation after three hours. The mean peak elevation of 102 mm. of water or 22.9 per cent and the total analgesic action of 70 per cent-hours were the lowest encountered.

*Acetylsalicylic acid:* Twelve patients were given 0.6 to 1.2 Gm. of acetylsalicylic acid by mouth (Table III). There was no variation from the control thresholds in any instance other than small changes in either direction usually found on repeated control determinations. Sodium salicylate given in comparable doses parenterally to four patients produced no greater changes in the pain threshold than those observed after parenteral administration of sterile saline solution. The composite time-action curve for seven patients (Figure 2) showed changes both above and below the control level which were within the limits of accuracy of the technique.

*Relationship of dosage to analgesia:* Four patients who were subjected to prolonged biliary drainage and whose control thresholds varied little from day to day received morphine and meperidine in increasing doses.

Time-action curves for one patient who received morphine and another after meperidine are shown in Figure 3. Increases in the lower dosage

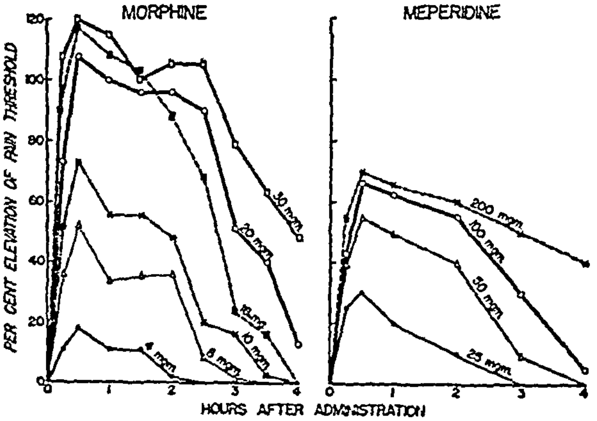

FIG. 3. TIME-ACTION CURVES IN PER CENT ELEVATION OF PAIN THRESHOLD FOLLOWING INCREASING DOSES OF MORPHINE AND MEPERIDINE

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[1] Metopon hydrochloride powder for parenteral use was obtained through the kindness of Dr. Nathan B. Eddy, Committee on Drug Addiction and Narcotics of the National Research Council.

TABLE V

*Relationship of dosage and visceral analgesia*

| Morphine sulfate | | | | Meperidine hydrochloride | | | |
|---|---|---|---|---|---|---|---|
| Dose, mgm. | Analgesia in per cent-hours | Dose ratio | Analgesia ratio | Dose, mgm. | Analgesia in per cent-hours | Dose ratio | Analgesia ratio |
| 4 | 22 | 1 | 1 | 25 | 43 | 1 | 1 |
| 8 | 86 | 2 | 4 | 50 | 114 | 2 | 2.7 |
| 10 | 140 | 2.5 | 6.4 | 100 | 172 | 4 | 4.1 |
| 16 | 269 | 4 | 12.4 | 200 | 239 | 8 | 5.6 |
| 20 | 301 | 5 | 13.9 | | | | |
| 30 | 329 | 7.5 | 15.2 | | | | |

ranges caused marked elevations of pain threshold. While 4 mgm. of morphine produced a peak threshold increase of 18 per cent, double the amount increased the peak to 52 per cent, and 16 mgm., again double the amount, raised the peak to 118 per cent above the control level. Further increases of the dose to 20 and 30 mgm. caused no higher threshold elevations than 16 mgm. The only effect of the largest doses was a prolongation of the action: no analgesic effect was noted at the end of four hours with doses up to 16 mgm. An appreciable threshold elevation was noted at the end of four hours with 20 mgm. and some action was detected after six hours with 30 mgm.

Time-action curves with increasing doses of meperidine showed a similar pattern of response. Doubling the dose from 25 to 50 mgm. caused a twofold increase of peak threshold elevation. Doubling the dose from 100 to 200 mgm. caused no increase of the peak action but prolonged the action considerably beyond four hours.

Dosages with corresponding per cent-hours and ratio of dosage with ratio of per cent-hours are shown in Table V. Increases in the lower ranges of morphine caused relatively much greater total analgesic action. Doubling the dose of 4 mgm. produced a fourfold increase of total analgesia and doubling the dose from 8 to 16 mgm. tripled the analgesic action. Further increases were relatively ineffective. Doubling 16 mgm. increased the total analgesic action by only 22 per cent from 269 to 329 per cent-hours. Increasing quantities of meperidine caused closely corresponding increases of total analgesic action up to 100 mgm. Double this last amount caused a further increase of analgesia of only 39 per cent. Actual values of the two studies could not be compared since they were done on different patients with different initial pain thresholds. In spite of our limiting pressures to 1,000 mm. of water, it was never possible to obliterate the pain sensation even with the largest doses of morphine.

*Relationship of level of initial pain threshold and the analgesic effect:* It was shown that in different patients the initial pain threshold varied from 90 to 800 mm. of water (Table I). It soon became apparent that in patients with initially low thresholds a relatively greater rise in pain threshold from analgesic drugs could be obtained than in patients with initially high thresholds. This was true whether the threshold raising action was expressed as elevation in mm. of water or as per cent elevation.

Initial pain thresholds and peak pain thresholds after 10 mgm. of morphine of 40 individual time-action curves are shown in Figure 4. With initial thresholds of 100 to 300 mm. of water, elevations due to morphine by 400 and even 500 mm. were

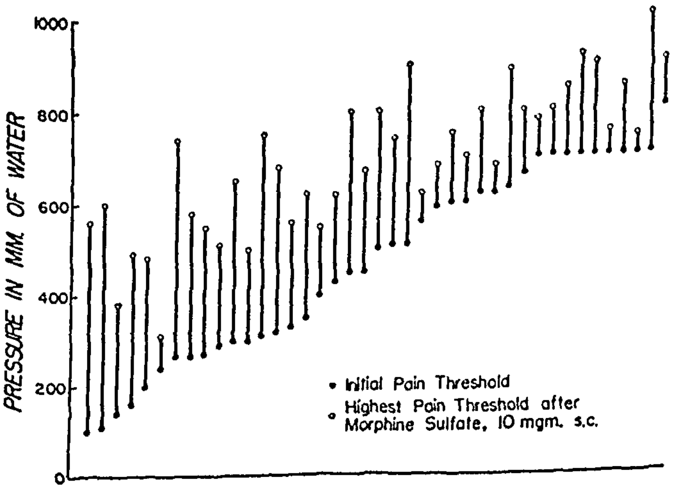

FIG. 4. PEAK THRESHOLD ELEVATIONS IN MM. OF WATER DUE TO 10 MGM. OF MORPHINE

Results of 40 studies arranged in increasing order of initial (control) thresholds.

not uncommon. With the highest initial thresholds of 500 to 700 mm. of water, threshold elevations due to morphine were considerably lower. Many were less than 100 mm. and most not over 200 mm. The mean threshold elevation of nine patients with initial thresholds between 90 and 290 mm. was 340 mm. The mean elevation of 16 patients with initial thresholds of 600 to 800 mm. was 125 mm.

This same phenomenon observed in a single patient who was studied with 100 mgm. meperidine from two to four weeks after operation is illustrated in Figure 5. Two weeks after operation, the initial pain threshold of 320 mm. rose to a peak of 550 mm. after meperidine. This rise of 230 mm. represents a 72 per cent elevation as indicated by the arrow. Further studies 17, 18 and 21 days after operation showed slowly increasing initial pain thresholds and a decreasing effect of the same dose of meperidine. The initial pain threshold 28 days after operation had risen to 600 mm. The increase to 670 mm. after meperidine represented only a 12 per cent elevation of pain threshold.

Patients were further grouped according to their initial pain threshold level and the elevation of threshold due to morphine, 10 mgm., meperidine, 100 mgm., and codeine, 60 mgm., were placed in the various groups (Figure 6). Other analgesics were omitted because of an insufficient number of patients in each group. Here, again, as in individual patients, there was a marked decrease of threshold-elevating activity with control thresholds of increasing magnitude. In the group of patients with control pain thresholds of 90–290 mm., morphine caused a mean rise of pain thresholds of 340 mm., meperidine of 130 mm. and codeine of 100 mm. In the last group with initial thresholds from 700 to 1,000 mm., the three elevations due to morphine, meperidine and codeine were 125, 50 and 40 mm. of water. Narcotics were more effective both relatively and absolutely if the initial visceral pain threshold was low because of inflammation. If the presumption that the early low thresholds are due to cholangitis is correct these findings show a good confirmation of observations made by Schumacher with thermal radiation on inflamed skin (23).

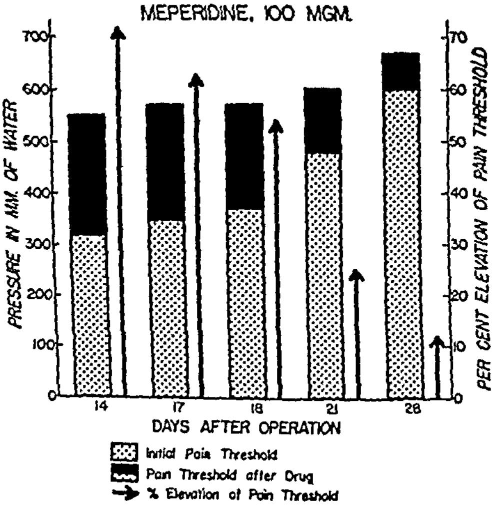

Fig. 5. Initial Pain Thresholds and Peak Thresholds in Mm. of Water after 100 Mgm. of Meperidine of one Patient Studied at Intervals from 14 to 28 Days after Operation

Arrows indicate per cent pain threshold elevation due to the medication.

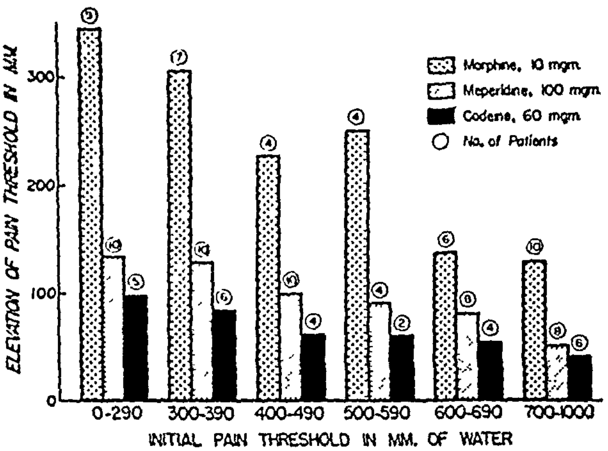

Fig. 6. Peak Pain Threshold Elevations in Mm. of Water following Morphine, Meperidine and Codeine, Grouped According to Magnitude of Initial (Control) Pain Threshold

*Placebos and the visceral pain threshold:* At the onset of these experiments it was suggested that the striking rise of the pain threshold after parenteral injection of analgesic drugs may in part be due to the psychologic effects of the injection. Patients were therefore chosen who had had several previous studies with large doses of potent analgesics. These studies were conducted in the usual manner except that after three initial threshold determinations 1 cc. of sterile saline solution was injected subcutaneously (Figure 7A). A very slight rise of the pain threshold (25 mm. or 8 per cent) was noted after 15 minutes and the

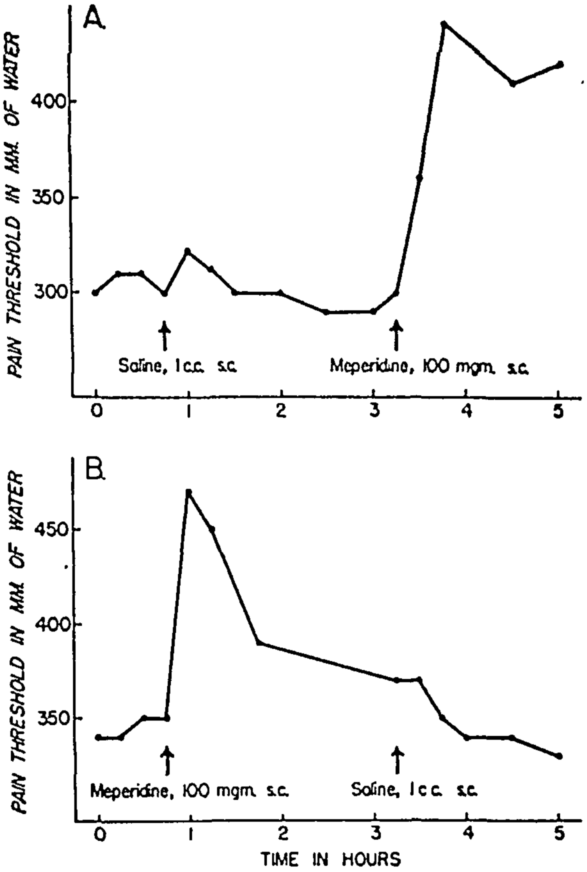

FIG. 7. THE EFFECT OF PARENTERAL PLACEBOS ON THE VISCERAL PAIN THRESHOLD

A. Saline solution followed in two and one half hours by meperidine; B. Meperidine and two and one half hours later saline solution.

threshold then returned to the control level and remained there for three hours. At the end of this time 100 mgm. meperidine were injected with a resultant pressure elevation of 70 mm. (23 per cent) after 15 minutes and 140 mm. (47 per cent) after 30 minutes (Figure 7A). Another patient was given 100 mgm. of meperidine and two and one half hours later, after the effect of the drug had nearly disappeared, 1 cc. of sterile saline solution (Figure 7B). Here there was no increase whatever in the pain threshold after the placebo but a further fall of the threshold as the last traces of the meperidine effect disappeared. From these and six other studies with similar results it was concluded that parenteral administration of placebos did not cause an elevation of the visceral pain threshold. Furthermore, increases of thresholds in suggestible patients were smaller than comparable threshold responses of skin pain (24).

*Relationship of visceral analgesia to visceral spasm:* The constipating effect of opium was utilized therapeutically many hundred years before its value as a central nervous system depressant was recognized. This effect is common to all the phenanthrene derivatives of opium and is in great part due to lessened motility and increased tone of the gastro-intestinal musculature. This spasmogenic activity on the gastro-intestinal and urinary tracts and the biliary tree constitutes an undesirable side effect in those instances where morphine and codeine are used for their narcotic effect. It was hoped that the appearance of synthetic analgesics would bring a solution to this problem. However, it was shown that both the new synthetic analgesics methadone and meperidine and the morphine derivatives dihydromorphinone and methyldihydromorphinone cause spasm of the small intestine and biliary tree of man (18, 19). The rise of intrabiliary resting pressure which invariably follows the administration of opiates is chiefly due to spasm of the second portion of the duodenum with occlusion of the sphincter of Oddi (16). The rise in pressure has been shown to be proportional to the severity of the spasm (18, 19).

The present method of visceral pain threshold

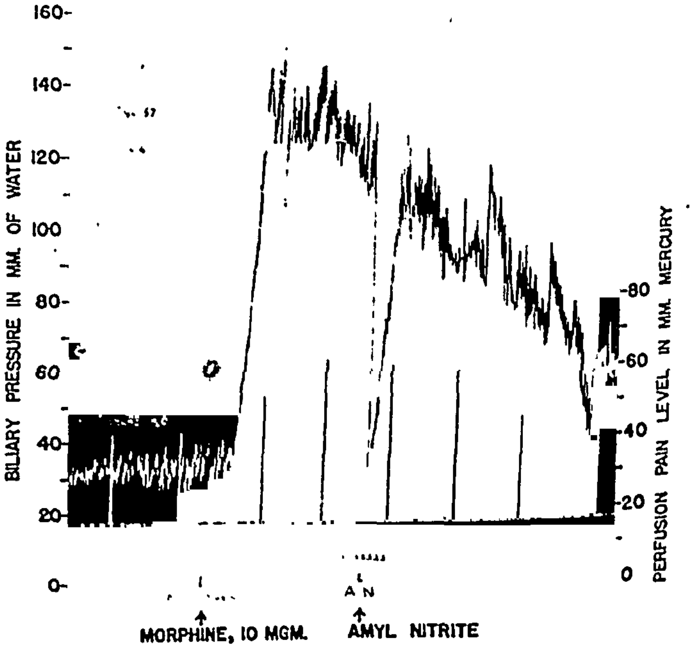

FIG. 8. KYMOGRAPH TRACING OF INTRABILIARY RESTING PRESSURE IN MM. OF WATER AND OF PAIN THRESHOLD IN MM. OF MERCURY FOLLOWING SUBCUTANEOUS INJECTION OF 10 MGM. OF MORPHINE

Three inhalations of amyl nitrite caused a prompt but brief fall in the intrabiliary pressure and had no effect on the pain threshold. The base line (0) is at the level of the xyphoid with the patient in the supine position. Time marker: three minutes.

determination offered a good opportunity for simultaneous quantitative estimation of visceral spasm.

Resting intrabiliary pressures were always recorded during intervals between pain threshold determinations. Occasionally pain threshold levels in mm. of mercury and resting pressures in mm. of water were recorded on the same smoked drum (Figure 8).

The onset of the analgesic effect was always accompanied by a gradual rise of intrabiliary pressure, and the elevation of this pressure was roughly proportional to the elevation of pain threshold (Figure 8). This applied to drugs of varying effectiveness as well as to the same drug given in varying doses. Figure 9 (top) shows the mean peak pain threshold elevations in mm. of water following injection of the six narcotics studied. The same figure (bottom) shows the peak resting pressure elevation after administration of these same drugs. The order of effectiveness of these drugs for both narcosis and spasmogenesis was the same and the ratio of effectiveness of the various agents was similar.

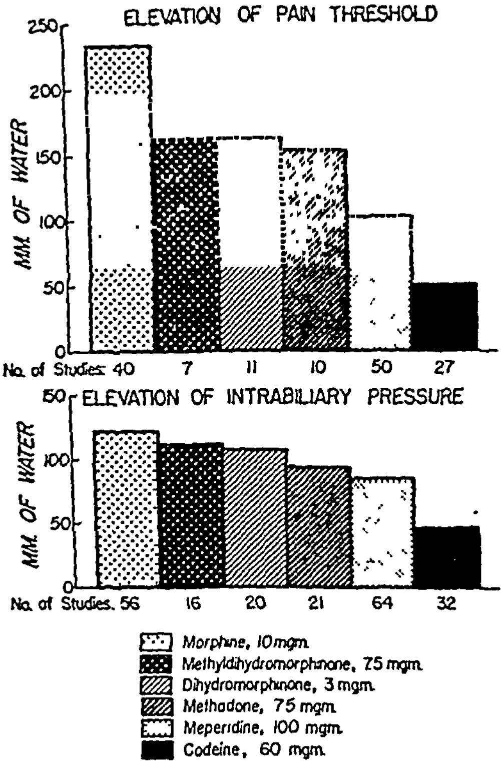

FIG. 9. MEAN PEAK PAIN THRESHOLD ELEVATIONS AND MEAN PEAK INTRABILIARY RESTING PRESSURES IN MM. OF WATER AFTER VARIOUS NARCOTICS

From these data, it is evident that every drug studied which possessed visceral analgesic qualities also caused visceral spasm. The reverse was true also: every drug which was shown to cause biliary and duodenal spasm also had analgesic properties. This is an unexpected and perhaps not significant finding. The analgesic qualities of these agents are due entirely to their action on the central nervous system while their spasmogenic effects are due entirely to local action on smooth muscle (25).

## DISCUSSION

Comparison of the effectiveness of various analgesic drugs is difficult because beneficial results depend to such a great extent on the dosage used. Frequently, doses of different agents, necessary to produce the same effect, have been compared. This is misleading since toxic effects of the drugs are thereby disregarded. The "saturation" level, or the highest threshold-raising effect of which a drug is capable (6) provides a good base line for comparison of analgesic effectiveness. This method of comparison was not available to us; first, because the dangers of overdistention of the common bile duct limited us to 1,000 mm. of water pressure, and second, because patient volunteers with common duct drainage, unlike healthy volunteers, are available for a limited time only. It was obvious from the onset that it would not be possible to study the same patients with all drugs and a great variety of dosage forms. We elected, therefore, to study all patients with as many drugs as possible and to confine studies, with a few exceptions, to only one dose and only one method of administration. The dose chosen was the average dose recommended by various sources for a 75 kg. man. The toxic effects of the amounts employed were roughly comparable: in patients without tolerance the dose was one-half of the upper limit of safe usage.

The peak analgesic action for visceral pain occurred earlier than for integumental pain with all drugs tested. The mean peak action for morphine and meperidine at the end of 30 minutes was found about one-half hour earlier than their maximal action determined by the von Frey and Hardy-Wolff-Goodell techniques, (6, 26). The other agents caused maximal relief of visceral pain 60 minutes after injection whereas their maximal effect on superficial pain was usually found after 90 minutes (26, 27). The measurable duration of action for visceral pain was about half that for skin pain. Wolff and associates found a return to the control threshold after seven hours with 8 mgm. morphine and 6.6 mgm. of methyldihydromorphinone (6). The short duration of action for visceral pain could not be attributed to a lesser overall effect of the drugs because of lower sensitivity of the method since the peak per cent elevation of skin pain due to radiant energy and of visceral pain due to distention were about the same.

Morphine was the most potent analgesic. The morphine derivatives were relatively four times as effective for superficial pain than for deep pain. Equivalent doses based on studies with the Hardy-Wolff-Goodell and von Frey techniques were 30 mgm. morphine for 3 mgm. dihydromorphinone or 6.6 mgm. methyldihydromorphinone (6, 26). The equivalent doses for visceral pain were roughly 8 mgm. of morphine for above doses of the morphine derivatives.

The effect of codeine on visceral pain also compares unfavorably with its action on skin pain. For the latter, the equivalent dose of morphine and codeine was 8:60 (6). For visceral pain this ratio was approximately 3:60.

Since the introduction of meperidine, the evaluation of its analgesic power has been scaled downward somewhat. At present, it is thought to be about twice as effective as codeine and much less effective than morphine (28). This position of meperidine among other analgesics was confirmed. The more rapid onset of action and the shorter duration of action may have been partly due to the fact that meperidine, unlike all other drugs, was injected intramuscularly.

Considerable controversy exists over the effectiveness and duration of action of methadone. Originally it was thought to be twice as effective as morphine both in man and experimental animals (29). Its investigation with the Wolff-Hardy-Goodell technique indicated that 5 mgm. was as potent as 15 mgm. of morphine (28). Clinical experience with methadone in the treatment of visceral pain revealed a somewhat lower potency. Troxil found it 1.5 times as active as morphine (30) and Denton and Beecher found no difference between methadone and morphine, mgm. for mgm., both with respect to speed of action and duration of effect (31). The present study confirmed methadone as a highly active analgesic agent. Present results differ in two respects from previous experience. From observation of patients, it was felt that 7.5 mgm. of methadone caused as much stupefaction, depression and drowsiness as 10 mgm. of morphine. This is in contrast to findings of others who observed no or only minimal sedation and narcosis (28, 29). Secondly, the duration of action of methadone was significantly greater than that of all other drugs tested. The shape of the time-action curve was therefore distinctly different. The total analgesic action of 7.5 mgm. methadone was 20 per cent greater than that of 10 mgm. morphine in spite of the latter's greater peak threshold raising ability.

The total lack of visceral analgesic activity of acetylsalicylic acid was striking. The long established clinical efficacy of acetylsalicylic acid in the treatment of headache, myalgia, arthralgia and other pain originating in integumental structures has been confirmed in the laboratory by quantitative skin threshold studies (32). A 35 per cent elevation of the skin pain threshold due to 0.3 Gm. of acetylsalicylic acid was equal to the effect of 45 mgm. of codeine. Harrison and Bigelow, using the ischemic muscle method, found a 25 per cent threshold elevation after 0.3 Gm. of acetylsalicylic acid (7). They concluded that acetylsalicylic acid is an effective analgesic agent for visceral pain. However, equally high and even higher threshold elevations were obtained with placebos. Furthermore, pain due to muscle ischemia should be grouped with pain from integumental structures and not with visceral pain.

Increasing doses of the same drug showed response patterns similar to those obtained from integumental structures. The "saturation" quantity or the smallest amount with which the highest threshold raising effect was obtained (6) was 16 mgm. for morphine and between 100 and 200 mgm. for meperidine. Both these values were somewhat smaller than those found for skin pain. The "saturation" level, or highest threshold raising effect of which the drug was capable (6) was 120 per cent for morphine and 70 per cent for meperidine—also somewhat lesser analgesic effects than those observed with skin pain.

## SUMMARY AND CONCLUSIONS

1. A quantitative method for measuring visceral pain thresholds by hydrostatic distention of the biliary ducts has been described. The method was used successfully in 32 of 40 patients who volunteered for these studies after surgical exploration of the common bile duct.

2. The painful sensation elicited by external elevation of the intrabiliary pressure was described as sharp or colicky by 47 per cent of the patients. The remainder reported dull pain or an ache. The pain was well localized in the right upper quadrant or epigastric region of the abdomen in 75 per cent of the patients and radiation to the back was noted by 25 per cent. For reliable end point determinations of the threshold the alertness of the patient at that time had to be assured.

3. The initial pain threshold in different patients varied from 90 to 800 mm. of water. A slow rise of pain threshold was often noted in the same patients studied repeatedly from 14 days to seven months after operation. However, all measurements of pain thresholds of the same patients on the same day were found to be at least within ± 10 per cent of their respective average values. In six patients studied at intervals during the course of 12 hours, the control threshold values showed a standard deviation of ± 5.1 to ± 12.6 mm. and a coefficient of variation of 0.67 to 3.82 per cent.

4. The administration of analgesic substances, with the exception of acetylsalicylic acid, invariably resulted in an elevation of the visceral pain threshold. Satisfactory time action curves were obtained on 157 occasions. The action of seven analgesic agents was studied. The doses used were: morphine, 10 mgm.; codeine, 60 mgm.; dihydromorphinone, 3 mgm.; methyldihydromorphinone, 7.5 mgm.; methadone, 7.5 mgm.; meperidine, 100 mgm.; and acetylsalicylic acid, 0.6 Gm.

5. The onset of action, shown by an elevation of the intrabiliary hydrostatic pressure tolerated without pain, was noted between seven and 15 minutes after injection with all drugs studied.

6. The peak threshold elevation was found 30 minutes after administration of morphine and meperidine and 60 minutes after all other agents. The mean peak threshold elevations in per cent together with the number of studies for each drug were as follows: morphine: 40 studies, 56 per cent, codeine: 27 studies, 12 per cent, dihydromorphinone: 11 studies, 42 per cent, methyldihydromorphinone: 7 studies, 46 per cent, methadone: 10 studies, 33 per cent, meperidine: 50 studies, 23 per cent, and acetylsalicylic acid: 13 studies, no threshold elevation.

7. The duration of action was five hours for morphine, four hours for codeine, dihydromorphinone and methyldihydromorphinone, six hours for methadone and three hours for meperidine.

8. The total analgesic action expressed by the area under the time-action curve in "per cent-hours" was 140 for morphine, 40 for codeine, 112 for dihydromorphinone, 98 for methyldihydromorphinone, 168 for methadone, 70 for meperidine and 0 for acetylsalicylic acid.

9. When morphine and meperidine were given in increasing doses, the total analgesic action increased in proportion to the dose up to 16 mgm. for morphine and 100 mgm. for meperidine. A further doubling of the dose to 32 and 200 mgm., respectively, caused only a 23 and 37 per cent respective increase in the total analgesic action.

10. The analgesic effect of various drugs was found to be inversely proportional to the initial pain threshold both in different patients and in the same patients studied at intervals.

11. Parenteral injections of placebos in eight patients were not reflected by a rise in the visceral pain threshold.

12. The severity of biliary and small intestinal spasm due to the analgesic agents studied was found to be in proportion to their analgesic activity.

13. Differences between visceral and integumental pain threshold responses to the various drugs were noted. The peak threshold-raising action for visceral pain occurred earlier than for superficial pain; the duration of action was shorter and the intensity of the analgesia was somewhat smaller for visceral pain. In the doses used, morphine showed the most powerful threshold raising activity and methadone showed the longest action. In contrast to integumental pain, acetylsalicylic acid had no analgesic activity for visceral pain.

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# RENAL FUNCTION AND ELECTROLYTE METABOLISM IN ACUTE GLOMERULONEPHRITIS[1]

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(Submitted for publication November 24, 1950; accepted, February 16, 1951)

Glomerular changes are perhaps the most outstanding anatomical lesions of acute glomerulonephritis. The glomerular tuft usually exhibits increased cellularity with deposits of fibrin or inflammatory exudate in the capsular space. The capsule and basement membranes may become thickened but the arterioles and renal tubules appear relatively normal in the early phases of the disease. Several papers describe a characteristic functional pattern for acute glomerulonephritis which reflects the dominant glomerular lesion (1–4).

The purpose of the present paper is to describe the relation of changes in renal functions to the development of the clinical manifestations of acute glomerulonephritis and of the exacerbation in chronic glomerulonephritis, especially in regard to water and electrolyte metabolism. The renal functions measured include glomerular filtration rate (GFR), effective renal plasma flow (ERPF) and maximum capacity of the renal tubules to excrete p-aminohippurate ($Tm_{PAH}$) or to reabsorb glucose ($Tm_G$).

## CASE MATERIAL

Facts relative to the diagnosis and onset of the disease for each patient are summarized in Table I. Elevated antistreptolysin titers (5) indicated a recent group A hemolytic streptococcal infection in 10 instances. Evidence of prior renal disease was absent in the eight patients classified as acute glomerulonephritis. Several may have had unrecognized chronic glomerulonephritis and their acute attack may have been an exacerbation rather than the initial attack. However, initial attacks seem likely in the five episodes that "healed"[2] and in the two patients whose urine and blood pressure were normal 16 and 35 days before the onset of signs and symptoms of renal disease. Four instances of exacerbation in chronic glomerulonephritis were observed, one of which occurred in patient 8, whose initial attack of acute glomerulonephritis was also studied. The criterion accepted for the diagnosis of exacerbation in chronic glomerulonephritis was "an abrupt and marked increase in the degree of hematuria" (8). Foreshortening of the latent period between infection and onset of symptoms characteristic of the exacerbation (8, 9) was noted in two of these patients.

The initial and final observations on the urine, blood pressure and edema for each patient are shown in Table II, along with the duration of the abnormalities. The final probable outcomes are indicated in the last column.[2]

The sudden development of nephrotic edema was not accepted as evidence either for the initial acute attack of glomerulonephritis or for an exacerbation in glomerulonephritis. At the time of observation, none of the patients had evidence of the nephrotic phase. Their serum albumin averaged 3.2 gm./100 ml. with a range of 2.5 to 4.5, while their serum cholesterol averaged 219 mg./100 ml. with a range of 179 to 294.

Signs and symptoms of congestive heart failure were present during the acute renal episode in only two patients (patients 9 and 11). These two patients suffered

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[1] Supported by a grant from the Life Insurance Medical Research Fund.

[2] Only relatively short follow-up periods were possible in the majority of the patients comprising this study. Thus, certain differentiation between complete healing and the development of chronicity following acute glomerulonephritis was difficult and sometimes impossible (6). However, patients 9, 10 and 11 were known to have chronic glomerulonephritis for one or more years prior to observation. Patient 8 was seen during his first attack and subsequently during an exacerbation. Patients 6 and 7 were seen periodically during one and one half and one years respectively after their initial attacks. During this time proteinuria persisted, a fact which supports the belief that the disease had probably become chronic. Patient 5 was followed for slightly more than one year after his acute glomerulonephritis. The last six urinalyses were normal, quite suggestive of healing. Patient 1 had three normal urines before loss for observation and likewise probably healed. Patients 2, 3 and 4 had three, three and two protein-free urines. However, minimal to moderate microscopic hematuria was still present at the last examination. Since microscopic hematuria may be one of the last abnormal signs to disappear during the healing of acute glomerulonephritis (7), these patients were classified as "probably healing," and included in the "healed" group in the subsequent discussions. Although a follow-up period of a year or more is necessary to differentiate with certainty between healing and chronicity, the presumptive outcomes in this group of patients are designated.

TABLE I

*Data concerned with the onset and diagnosis of acute episodes of glomerulonephritis*

| PATIENT NUMBER | SEX | AGE (years) | PRECEDING INFECTION | MAXIMUM ANTISTREPTOLYSIN TITER (units) | LATENT PERIOD (days) | ONSET NEPHRITIS |
|---|---|---|---|---|---|---|
| 1 | M | 53 | Discharge right nostril | 100 | ? | *Acute*<br>Edema eyes, feet<br>Scanty urine<br>Microscopic hematuria<br>Hypertension |
| 2 | M | 50 | Tonsillitis, Fever<br>Rx: Sulfadiazine | 2000 | 20 | *Acute*<br>Edema eyes, feet<br>Microscopic hematuria<br>Hypertension |
| 3 | M | 56 | R.U.L. Pneumonia<br>Rx: Penicillin<br>(pneumococci not demonstrated) | 700 | 19 | *Acute*<br>(Urine, BP normal 16 days before onset)<br>Edema eyes, feet<br>Microscopic hematuria<br>Hypertension |
| 4 | M | 35 | Severe sore throat | >2000 | | *Acute*<br>Edema legs<br>Microscopic hematuria<br>Hypertension |
| 5 | M | 43 | Chronic cough<br>"Frequent colds" | 300 | ? | *Acute*<br>Scanty dark urine<br>Edema legs<br>Hypertension |
| 6 | M | 47 | None noted | 200 | ? | *Acute*<br>Abdominal cramps<br>Smoky urine<br>Edema face, legs<br>Hypertension |
| 7 | F | 46 | Postop (cervix) Infection<br>Rx: Penicillin | 900 | 35<br>(postop) | *Acute*<br>(Urine, BP normal pre-op.)<br>Edema eyes, feet<br>Microscopic hematuria<br>Hypertension |
| 8, #1 | M | 46 | Fever, cough | 400 | 8 | *Acute*<br>Bloody urine<br>Edema eyes, feet<br>Hypertension |
| 8, #2 | | | Malaise | 500 | 0 | *Exacerbation*<br>Bloody urine<br>Edema eyes, feet<br>Hypertension |
| 9 | F | 56 | Sore throat | --- | 1 | *Exacerbation*<br>(disease known 1 year)<br>Edema feet<br>Flank pain<br>Red urine<br>Hypertension<br>Dyspnea |
| 10 | M | 63 | Cold, fever | 400 | 3 | *Exacerbation*<br>(Initial attack 22 years ago)<br>(Exacerbation 12 years ago)<br>Edema legs<br>Pain low back<br>Red urine |
| 11 | M | 52 | None noted | 400 | ? | *Exacerbation*<br>(disease known 15 years)<br>Dyspnea<br>Orthopnea<br>Edema<br>Oliguria |

TABLE II

*Initial and final observations on urine, edema and blood pressure*

| PATIENT NUMBER | OBSERVATION | URINE | | | EDEMA (MAXIMUM WT. LESS USUAL WT.) (lbs) | BLOOD PRESSURE (mm. Hg) | PROBABLE OUTCOME ** |
|---|---|---|---|---|---|---|---|
| | | PROTEIN | RBC | CASTS | | | |
| 1 | Admission | + + + + | 10/hpf* | 5/hpf | 36 | 180/90 | |
| | Final | 0 | 0 | 0 | 0 | 120/70 | Healed |
| | Duration | (- - - - - - 3½ weeks - - - - - -) | | | (3 weeks) | (3 weeks) | |
| 2 | Admission | + + | 50/hpf | 2/hpf | 10 | 210/110 | |
| | Final | 0 | 15/hpf | rare | 0 | 135/80 | Healing |
| | Duration | (- - - - - - 3½ weeks - - - - - -) | | | (2 weeks) | (2 weeks) | |
| 3 | Admission | + + + | 100/hpf | occ. | 8 | 130/100 | |
| | Final | 0 | 10/hpf | occ. | 0 | 110/80 | Healing |
| | Duration | (- - - - - - 6 weeks - - - - - -) | | | (3 weeks) | (3 weeks) | |
| 4 | Admission | + + | innumerable | occ. | 38 | 198/118 | |
| | Final | 0 | few | 0 | 0 | 155/90 | Healing |
| | Duration | (- - - - - - 7 weeks - - - - - -) | | | (6 weeks) | (9 weeks) | |
| 5 | Admission | + | innumerable | rare | 21 | 144/90 | |
| | Final | 0 | 0 | 0 | 0 | 124/70 | Healed |
| | Duration | (- - - - - - 17 weeks - - - - - -) | | | (5½ weeks) | (10 weeks) | |
| 6 | Admission | + + + | gross | many | 33 | 165/100 | |
| | Final | + | 0 | 0 | 0 | 125/85 | Chronic |
| | Duration | (1½ yrs.+) | (- - 12 weeks - - ) | | (5 weeks) | (5 weeks) | |
| 7 | Admission | + + + | 10/hpf | occ. | 22 | 245/110 | |
| | Final | + + | 0 | 0 | 0 | 130/75 | Chronic |
| | Duration | (persisted) | (- - 10 weeks - - ) | | (5 weeks) | (9 weeks) | |
| 8, #1 | Admission | + + + + | gross | 3/lpf | 21 | 160/110 | |
| | Final | + + + | many | 0 | 0 | 130/84 | Chronic |
| | Duration | (- - - - - persisted - - - - - - ) | | | (3 weeks) | (3½ weeks) | |
| 8, #2 | Admission | + + + + | gross | occ. | 10 | 160/100 | |
| | Final | + + | 20/hpf | 0 | 0 | 130/90 | Chronic |
| | Duration | (- - - - - persisted - - - - - - ) | | | (4 weeks) | (3 weeks) | |
| 9 | Admission | + + + | gross | occ. | 18 | 210/120 | |
| | Final | + + + | 0 | 0 | 0 | 140/80 | Chronic |
| | Duration | (persisted) | (- - 4 weeks - - ) | | (2½ weeks) | (3 weeks) | |
| 10 | Admission | + + + | gross | many | 53 | 190/110 | |
| | Final | + + | 15/hpf | 0 | 0 | 180/100 | Chronic |
| | Duration | (- - - - - persisted - - - - - - ) | | | (6 weeks) | (persisted) | |
| 11 | Admission | + + + + | gross | occ. | 31 | 188/120 | |
| | Final | + + | 10/hpf | rare | 0 | 146/84 | Chronic |
| | Duration | (- - - - - persisted - - - - - - ) | | | (10 weeks) | (14 weeks) | |

* h.p.f. = high power field

** see footnote 2

from dyspnea and orthopnea, had elevated venous pressures and prolonged circulation time. Their hearts were enlarged.

## METHODS

All data presented represent an average of three or more consecutive 15 to 30 minute periods. All observations were performed in the morning. Fluids and food were withheld after the preceding evening meal. Following three periods during which inulin and PAH clearances and electrolyte excretion were observed, Tm glucose or $Tm_{PAH}$ were measured for three consecutive periods. Priming and sustaining infusions containing inulin and PAH were made up in distilled water. The sustaining infusion was delivered by means of a pump at the rate of 1 ml./minute during the first three periods, and at a rate between 2 and 5 ml./minute during Tm measurements. An arterial blood sample was obtained at the midpoint of each urine collection. Plasma, separated from the erythrocytes within 15 minutes of withdrawal of the blood sample, was used for the potassium, chloride, inorganic phosphate, inulin, PAH and glucose determinations. Dried heparin and a small amount of sodium fluoride were used to prevent clotting and glycolysis in the case of glucose and inorganic phosphate, while dried heparin alone was used to prevent clotting in the sample subjected to the other analyses. Serum was used for the sodium determinations.

Inulin was measured by Harrison's (10) modification of the method of Alving, Rubin and Miller (11), PAH by the Bratton-Marshall reaction (12), and glucose by a modification (13) of the Folin method. Sodium in the serum and urine and potassium in the plasma and urine were measured on an internal standard flame photometer. Chlorides were measured by a modification of the Volhard-Harvey method (14), inorganic phosphate by the method of Fiske and SubbaRow (15), and ammonia and urea by Summerson's modification (16) of the Van Slyke and Cullen procedure. Urine pH was determined with the glass electrode.

## *RESULTS*

### (*A*) *Renal functions*

Renal functions, weight, blood pressure and urinary abnormalities are recorded in Table III in relation to time of onset and of diuresis. The renal function values are not corrected to a standard surface area because of the complications this would introduce in the subsequent considerations of electrolyte excretions. Glomerular filtration rate was definitely reduced at the first observation in all but one instance (patient 2). The renal plasma flow[3] was less affected and was in the normal range at the first observation in four of the eight initial attacks of acute glomerulonephritis. The pattern of renal dysfunction in both initial attacks and in exacerbations was characteristic of that previously described for acute glomerulonephritis. The filtration fraction (GFR/RPF) was reduced (less than the mean normal value minus two standard deviations) at some time in eight of the 12 episodes. $Tm_G$ was reduced in eight of the nine instances in which it was measured, and $Tm_{PAH}$ in seven of nine instances. The ratios of filtration rate to $Tm_G$ and $Tm_{PAH}$ were usually reduced, indicating a greater impairment of glomerular activity than of tubular function. Several exceptions, however, were noted. In the instance of the four exacerbations the renal functions were undoubtedly abnormal prior to the observations because of the chronic renal disease but exhibited the same general pattern as the initial attacks.

Following the acute episode all functions returned toward normal in patients suffering either their first attack or an exacerbation. $Tm_G$ and $Tm_{PAH}$ were in the normal range at the time of the last observation in four of the five patients who healed or were probably healing (see footnote 2) while the filtration rate had returned to low normal values in three. These findings are similar to those reported in an earlier paper (1) which included observations on five patients suffering their initial attack of glomerulonephritis and two undergoing exacerbations. Serial observations on eight patients in the present study and five patients in the earlier study (1) are available in whom the outcome of the initial attack of acute glomerulonephritis was known (see footnote 2). The first estimation of renal function was obtained within two weeks or less of the onset of the disease in only five of the 13 patients. The serial filtration rates are presented in Figure 1. In general, a smaller reduction in filtration rate and a more rapid return to normal occurred among the six patients whose nephritis healed or probably healed than among the seven whose disease became chronic. Of the nine patients studied within four weeks of the onset of nephritis, the three whose disease failed to heal had initial filtration rates below 55 ml./minute, while those who healed had rates of 44,

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[3] The renal plasma flow values in this study are not corrected for any possible incomplete extraction of PAH by the renal tubules. The true renal plasma flow in any instance where there is incomplete extraction of PAH is, therefore, higher than the recorded value.

TABLE III

*Blood pressure, urine and renal function tests in patients with acute glomerulonephritis and with exacerbations in chronic glomerulonephritis*

| Patient number | Days after onset | Relation to diuresis | Weight (lbs) | Blood pressure (mm. Hg) | Urine | | Concentration test (sp. gr.) | Blood urea (mg/100 ml) | Glomerular filtration rate (ml/min) | Renal plasma flow (ml/min) | Filtration fraction (percent) | $Tm_{PAH}$ (mg/min) | $Tm_G$ (mg/min) | $GF/Tm_{PAH}$ | $GF/Tm_G$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Protein | RBC | | | | | | | | | |
| Ave Normal Male (17) | | | | | | | | | 131 | 697 | 19.0 | 76.1 | 375 | 1.72 | 0.371 |
| σ | | | | | | | | | 21.5 | 136 | 2.4 | ---- | 79.7 | ---- | 0.0563 |
| 1 | 8 | 1 day before | 184 | 194/90 | 3+ | 10/hpf* | 1.012 | 65.0 | 82.6 | 551 | 15.0 | 52.2 | ----- | 1.38 | ------ |
| | 16 | Last day | 158 | 164/86 | 0 | 0 | 1.012 | 23.0 | 78.0 | 587 | 13.3 | 66.1 | ----- | 1.06 | ------ |
| | 30 | 13 days after | 154 | 120/70 | 0 | 0 | 1.020 | ---- | 106 | 609 | 17.4 | 62.1 | ----- | 1.71 | ------ |
| 2 | 7 | First day | 151 | 160/110 | 2+ | 20/hpf | 1.017 | 94.7 | 100 | 584 | 17.1 | ---- | 377 | ---- | 0.27 |
| | 21 | 7 days after | 146 | 130/78 | 1+ | 20/hpf | 1.017 | 20.7 | 102 | 565 | 18.1 | ---- | 374 | ---- | 0.27 |
| 3 | 12 | 3 days before | 151 | 130/90 | 2+ | 50/hpf | 1.015 | 50.1 | 78.0 | 369 | 21.1 | ---- | 241 | ---- | 0.32 |
| | 22 | 2 days after | 144 | 118/70 | 1+ | 10/hpf | 1.018 | 50.3 | 73.4 | 369 | 19.9 | 57.7 | ----- | 1.27 | ------ |
| | 43 | 23 days after | 148 | 110/80 | 0 | 15/hpf | ----- | 45.9 | 73.7 | 384 | 19.2 | ---- | 199 | ---- | 0.37 |
| 4 | 26 | 1 days before | 169 | 170/110 | 2+ | innumerable | 1.011 | 48.0 | 43.5 | 613 | 7.1 | ---- | 184 | ---- | 0.24 |
| | 30 | 3rd day during | 161 | 178/110 | 3+ | innumerable | ----- | 36.3 | 43.6 | 481 | 9.1 | 18.5 | ----- | 2.38 | ------ |
| | 52 | 8 days after | 136 | 155/80 | 0 | occ. | 1.011 | ---- | 47.7 | 357 | 13.4 | 20.1 | ----- | 2.39 | ------ |
| 5 | 34 | 4th day during | 128 | 144/90 | 1+ | 30/hpf | 1.010 | 30.0 | 58.9 | 331 | 17.8 | 41.6 | ----- | 1.41 | ------ |
| | 43 | 4 days after | 126 | 148/86 | 0 | 10/hpf | 1.011 | 30.0 | 64.8 | --- | ---- | ---- | 248 | ---- | 0.26 |
| | 63 | 24 days after | 140 | 148/86 | 0 | 5/hpf | ----- | 24.0 | 93.4 | --- | ---- | ---- | 333 | ---- | 0.28 |
| | 118 | 3 months after | 138 | 124/70 | trace | 0 | 1.015 | 17.4 | 94.0 | 526 | 17.8 | ---- | 353 | ---- | 0.27 |
| | 206 | 6 months after | 150 | 120/70 | 0 | 0 | ----- | 28.4 | 104 | 489 | 21.3 | 82.0 | ----- | 1.27 | ------ |
| 6 | 11 | 3 days before | 214 | 165/95 | 2+ | 30/hpf | 1.014 | 90.0 | 32.6 | 458 | 7.1 | 34.3 | ----- | 0.88 | ------ |
| | 20 | 7th day during | 193 | 154/90 | 1+ | 200/hpf | 1.010 | 61.5 | 41.4 | 328 | 12.6 | ---- | 129 | ---- | 0.32 |
| | 76 | 5 weeks after | 181 | 125/85 | 1+ | 0 | ----- | 26.2 | 84.8 | 442 | 19.2 | ---- | 227 | ---- | 0.37 |
| 7 | 22 | 1 day before | 177 | 220/98 | 3+ | 5/hpf | 1.008 | 33.7 | 38.5 | 282 | 13.7 | ---- | 165 | ---- | 0.23 |
| | 36 | Last day during | 146 | 154/80 | 2+ | 0 | ----- | 47.4 | 37.0 | 279 | 13.3 | 35.8 | ----- | 1.04 | ------ |
| | 54 | 18 days after | 148 | 130/75 | 2+ | 0 | ----- | 56.3 | 47.7 | 312 | 15.3 | ---- | ----- | ---- | ------ |
| 8, #1 | 12 | 2nd day during | 138 | 140/90 | 4+ | 30/hpf | 1.016 | 46.6 | 54.9 | 523 | 10.5 | ---- | 133 | ---- | 0.41 |
| | 22 | Last day during | 122 | 140/90 | 4+ | innumerable | 1.017 | 49.8 | 48.7 | 444 | 11.0 | ---- | 116 | ---- | 0.43 |
| | 33 | 11 days after | 128 | 115/80 | 3+ | many | ----- | 57.2 | 33.3 | 234 | 14.3 | 15.4 | ----- | 2.16 | ------ |
| | 48 | 26 days after | 126 | 130/84 | 3+ | occ. | ----- | 53.4 | 35.8 | 260 | 13.8 | ---- | 108 | ---- | 0.40 |
| 8, #2 | 12 | 4 days before | 141 | 176/110 | 4+ | innumerable | 1.015 | 53.0 | 27.0 | 117 | 23.1 | 6.7 | ----- | 4.03 | ------ |
| | 27 | 1 day after | 131 | 120/74 | 3+ | 25/hpf | ----- | 45.0 | 41.4 | 386 | 10.7 | 25.1 | ----- | 1.65 | ------ |
| 9 | 17 | 4th day during | 147 | 150/90 | 2+ | 4/hpf | 1.016 | 37.8 | 61.7 | 356 | 17.3 | ---- | 228 | ---- | 0.27 |
| | 28 | 9 days after | 147 | 148/76 | 3+ | 0 | ----- | 28.7 | 74.6 | 348 | 21.4 | ---- | 269 | ---- | 0.28 |
| 10 | 16 | 3rd day during | 182 | 175/105 | 3+ | innumerable | 1.015 | 40.6 | 45.0 | 280 | 16.1 | ---- | 115 | ---- | 0.39 |
| | 35 | 22nd day during | 156 | 180/100 | 3+ | 15/hpf | ----- | 33.6 | 38.6 | 277 | 14.0 | ---- | 125 | ---- | 0.31 |
| 11 | 15 | 3rd day during | 149 | 176/110 | 3+ | 30/hpf | ----- | 110 | 21.9 | 351 | 6.2 | 40.8 | ----- | 0.54 | ------ |
| | 40 | 28th day during | 149 | 144/100 | 3+ | 20/hpf | ----- | 59.6 | 32.9 | 394 | 8.4 | 41.0 | ----- | 0.80 | ------ |
| | 67 | 55th day during | 131 | 174/98 | 2+ | 10/hpf | ----- | 27.5 | 54.4 | 424 | 12.8 | 46.8 | ----- | 1.16 | ------ |
| | 98 | 22nd day after | 136 | 162/110 | 2+ | occ. | ----- | 26.7 | 41.7 | 220 | 19.0 | 40.7 | ----- | 1.02 | ------ |
| | 117 | 41st day after | 135 | 150/94 | 2+ | 40/hpf | ----- | 33.2 | 55.5 | 370 | 15.0 | ---- | ----- | ---- | ------ |

* h.p.f. = per high power field =

Each renal function datum represents an average of three consecutive periods. Glomerular filtration rates used in calculating GFR to Tm ratios were those measured prior to infusion of large amounts of glucose or PAH. Concentration tests recorded represent the maximum specific gravity attained after a 12 hour restriction of fluid, and were performed within one week of the time indicated.

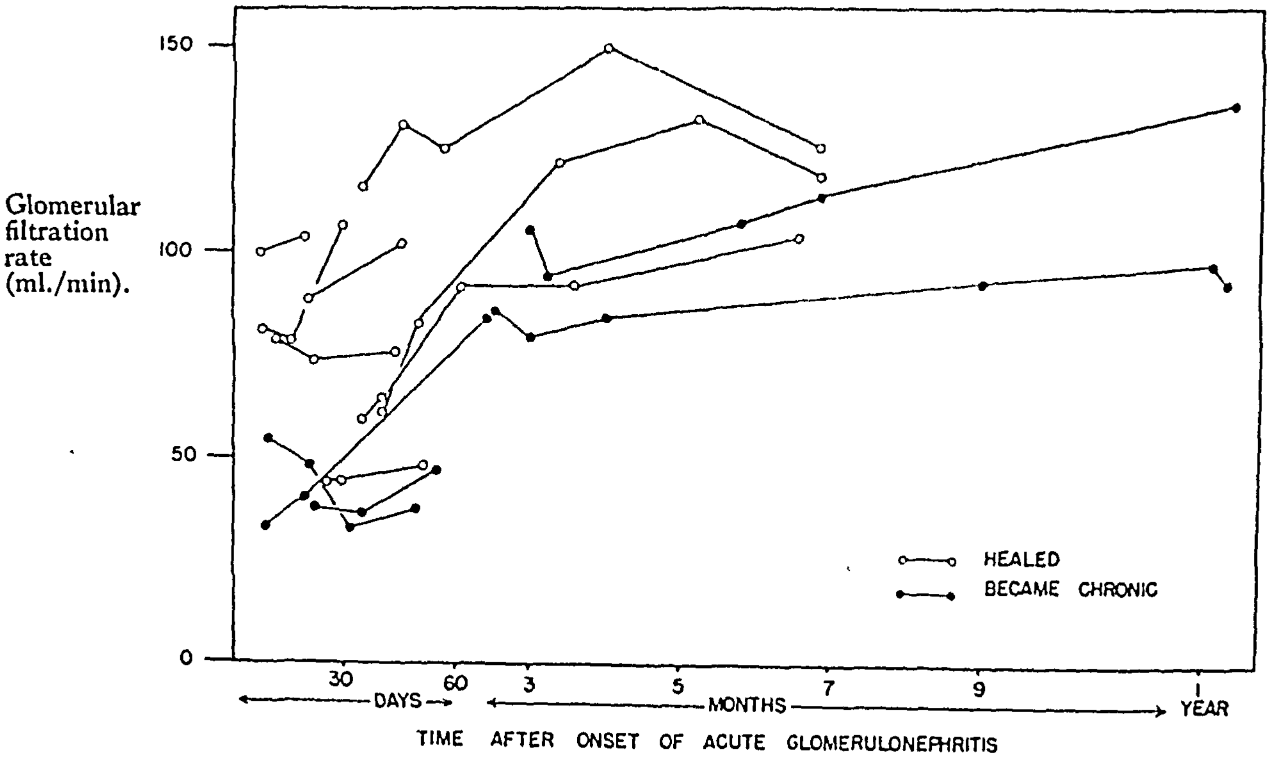


FIG. 1. GLOMERULAR FILTRATION RATE IN RELATION TO TIME AFTER ONSET OF ACUTE GLOMERULONEPHRITIS

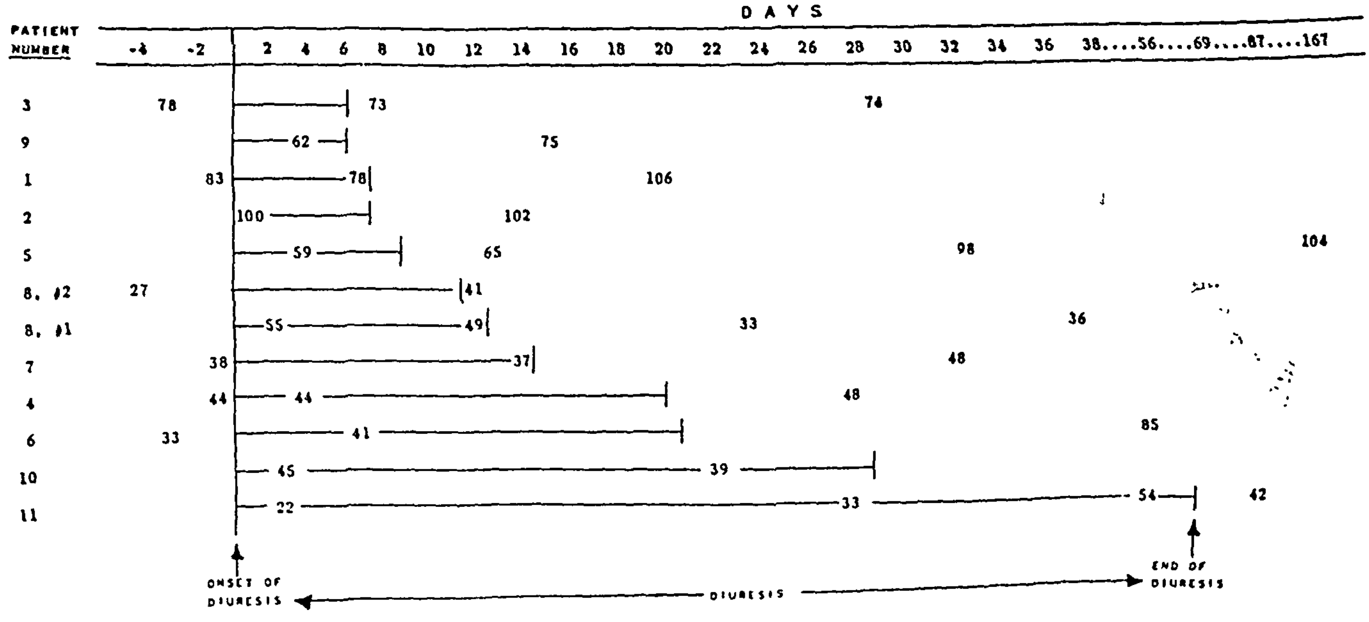
59, 81, 87 and 100 ml./minute. Return of filtration rate to the normal range, however, was not incompatible with the development of chronic renal disease, as in patients 4 and 9 of the earlier study (1). Conversely, patient 6 of the early study and patient 5 of the present study healed completely in spite of rather slow improvement in their glomerular filtration rates. Changes in Tm values showed the same general trends.

Concerning the inter-relations of the several renal functions, the filtration fraction usually increased as the acute renal process improved. However, no correlation could be established between this function or the ratio of filtration rate to excretory or reabsorptive Tm and the eventual outcome.

The exacerbations in chronic glomerulonephritis suffered by patients 9 and 11 were complicated

**TABLE IV**

*Glomerular filtration rate in relation to onset and end of diuresis in acute glomerulonephritis*



| PATIENT NUMBER | -4 | -2 | 2 | 4 | 6 | 8 | 10 | 12 | 14 | 16 | 18 | 20 | 22 | 24 | 26 | 28 | 30 | 32 | 34 | 36 | 38 | 56 | 69 | 87 | 167 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 3 |  | 78 |  |  |  | 73 |  |  |  |  |  |  |  |  |  | 74 |  |  |  |  |  |  |  |  |  |
| 9 |  |  |  | 62 |  |  |  |  |  | 75 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 1 |  | 83 |  |  | 78 |  |  |  |  |  |  | 106 |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 2 |  |  | 100 |  |  |  |  |  | 102 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 5 |  |  |  | 59 |  |  |  | 65 |  |  |  |  |  |  |  |  |  | 98 |  |  |  |  |  |  | 104 |
| 8, #2 | 27 |  |  |  |  |  |  | 41 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 8, #1 |  |  | 55 |  |  |  |  | 49 |  |  |  |  |  | 33 |  |  |  |  |  |  | 36 |  |  |  |  |
| 7 |  | 38 |  |  |  |  |  |  | 37 |  |  |  |  |  |  |  |  | 48 |  |  |  |  |  |  |  |
| 4 |  | 44 |  | 44 |  |  |  |  |  |  |  |  |  |  |  | 48 |  |  |  |  |  |  |  |  |  |
| 6 |  | 33 |  |  | 41 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 85 |  |  |  |
| 10 |  |  |  | 45 |  |  |  |  |  |  |  |  | 39 |  |  |  |  |  |  |  |  |  |  |  |  |
| 11 |  |  |  | 22 |  |  |  |  |  |  |  |  |  |  |  | 33 |  |  |  |  |  | 54 |  | 42 |  |

D A Y S
38....56....69....87....167
ONSET OF DIURESIS
DIURESIS
END OF DIURESIS

by congestive heart failure, as evidenced by dyspnea, orthopnea, increased venous pressure, prolonged circulation time and enlargement of the heart. Nevertheless, the functional changes were characteristic of acute glomerulonephritis with a low filtration fraction, rather than that of congestive heart failure with an elevated filtration fraction. Four other patients had slight transient cardiac enlargement by X-ray examination, but no other signs or symptoms suggestive of heart failure. All patients had hypertension at the time of the first measurement of renal function. In five the diastolic blood pressure was 100 mm. Hg or more. In spite of the consistent hypertension, the filtration fraction was greater than the mean normal in only two instances. The blood pressure often returned to normal well before the renal function reached its maximum observed value.

### (*B*) *Relation of discrete renal functions to diuresis in the recovery phase of acute glomerulonephritis*

The relation of glomerular filtration rate to diuresis in acute episodes of glomerulonephritis is indicated in Table IV. Daily excretion of electrolytes was followed in four of the patients (pa-

TABLE V

*The relation of urea clearance to glomerular filtration rate and diuresis in acute glomerulonephritis*

| PATIENT | RELATION TO DIURESIS | URINE FLOW (ml./min) | GLOMERULAR FILTRATION RATE (GFR) (ml./min.) | INULIN U/P RATIO | UREA CLEARANCE (UC) (ml./min.) | UC/GFR | PLASMA UREA (mg./100 ml) |
|---|---|---|---|---|---|---|---|
| 1 | 1 day before | 1.87 | 82.6 | 44.1 | 35.9 | 0.44 | 65.0 |
| | last day during | 1.46 | 78.0 | 53.5 | 66.7 | 0.86 | 23.0 |
| 2 | 1st day during | 6.09 | 100 | 16.4 | 28.0 | 0.28 | 94.7 |
| | 7 days after | 4.07 | 102 | 24.5 | 80.0 | 0.79 | 20.7 |
| 3 | 3 days before | 2.69 | 78.0 | 29.0 | 42.2 | 0.54 | 42.2 |
| | 2 days after | 1.72 | 73.4 | 42.6 | 39.4 | 0.54 | 39.4 |
| | 23 days after | 2.62 | 73.7 | 28.1 | 43.3 | 0.59 | 43.3 |
| 4 | 3 days before | 1.30 | 43.5 | 33.4 | 25.7 | 0.59 | 48.0 |
| | 2 days after | 1.75 | 43.6 | 25.0 | 36.3 | 0.83 | 36.3 |
| 6 | 7th day during | 2.27 | 41.4 | 18.2 | 22.0 | 0.53 | 61.5 |
| | 5 weeks after | 1.92 | 84.8 | 44.2 | 71.8 | 0.85 | 26.2 |
| 7 | 1 day before | 2.29 | 38.5 | 16.8 | 26.4 | 0.69 | 33.7 |
| | last day during | 1.24 | 37.0 | 29.8 | 19.5 | 0.53 | 47.4 |
| | 18 days after | 2.21 | 47.7 | 21.6 | 28.6 | 0.60 | 56.3 |
| 8, #1 | 2nd day during | 1.93 | 54.9 | 28.4 | 35.6 | 0.65 | 46.6 |
| | last day during | 0.82 | 48.7 | 55.5 | 24.1 | 0.49 | 49.8 |
| | 11 days after | 2.62 | 33.3 | 12.7 | 26.6 | 0.80 | 57.2 |
| | 26 days after | 2.96 | 35.8 | 12.1 | 22.9 | 0.64 | 53.4 |
| 9 | 4th day during | 0.62 | 61.7 | 99.5 | 16.3 | 0.27 | 37.8 |
| | 9 days after | 1.02 | 74.6 | 73.3 | 31.2 | 0.42 | 28.7 |
| 10 | 3rd day during | 1.57 | 45.0 | 28.7 | 25.6 | 0.57 | 40.6 |
| | 22nd day during | 1.26 | 38.6 | 30.7 | 26.1 | 0.68 | 33.6 |
| 11 | 3rd day during | 0.96 | 21.9 | 22.8 | 9.4 | 0.43 | 110 |
| | 28th day during | 0.91 | 32.9 | 36.1 | 15.8 | 0.48 | 59.6 |
| | 55th day during | 3.18 | 54.4 | 17.1 | 32.0 | 0.59 | 27.5 |
| | 22nd day after | 1.57 | 41.7 | 26.6 | 27.5 | 0.66 | 26.7 |
| | 41st day after | 1.57 | 55.5 | 36.4 | 28.5 | 0.51 | 33.2 |

Each datum represents the average of three consecutive periods.

tients 1, 2, 4 and 6). In the remaining patients the weight curve and clinical evidences of edema were utilized to assess diuresis. Filtration rates were measured before the beginning of diuresis in six instances. In the remaining six instances, filtration rate was first observed on the first to fourth day of diuresis (within a few hours of the onset of diuresis in patient 2). The data recorded in Table IV indicate that diuresis following acute glomerulonephritis is not accompanied by a consistent change in filtration rate.

Urea clearance was measured concomitantly with glomerular filtration rate in most of the studies (Table V). The relation of urea clearance to filtration rate and urine flow was not consistent when the group of patients with acute nephritis was considered as a whole. Considerable increases in urea clearance, however, occurred in five patients whose course was characterized by rapid loss of edema in the early phase of diuresis (patients 1, 2, 4, 6 and 9). In three of these the urea clearance increased with no change in glomerular filtration rate, and in each a definite rise in the ratio of urea/inulin ratio occurred. Nor were the increases in urea clearance attributable to concomitant changes in urine flow, which showed only minor variations among the observations obtained for a given patient.

In general, the plasma urea concentration reflected change in urea clearance, and in turn, the filtration rate. Abnormal plasma urea levels, however, did occur in several instances where the filtration rate was not greatly reduced (patients 1–3), while an increasing filtration rate was not incompatible with an increasing plasma urea (patient 7).

In a number of the observations the urea clearance was measured both before and during the infusion of glucose or PAH necessary for the Tm measurements. Urine flow increased in 25 of 27 such observations among 10 patients. The urea clearance increased more than 20 per cent in 11 instances, while the urea/inulin clearance ratio increased in 20 of the 27 observations. In general, the greatest increases were noted among those patients whose urine flows were low during the control periods and who showed substantial diuresis during the osmotic loads. These data indicate that the urea clearance may be influenced by osmotic diuresis in patients with acute glomerulonephritis. The response of these patients to osmotic load was not unlike that observed among patients without renal disease (18).

### (C) *Effects of acute glomerulonephritis on electrolyte metabolism*

Observations on the plasma or serum levels and the excretion rates of sodium, potassium, chloride, and inorganic phosphate are recorded in Table VI. Data on the excretion of ammonia and urinary pH, and for reference purposes, concurrent filtration rates and urine flows, are included. The fraction of electrolyte filtered at the glomeruli that appeared in the urine also was calculated.

(*1*) *Sodium:* Serum sodium levels were usually in the normal range, although values between 132 and 135 meq./L were observed on occasion in six of the 12 acute episodes of glomerulonephritis. No correlation between the serum sodium level and the presence or absence of edema was noted. Sodium excretion, measured during the determination of filtration rate, was generally greater during the phase of diuresis than at other times. However, the necessity of giving intravenous infusions for clearance purposes probably distorted sodium excretion and urine flow to some extent, limiting the value of such observations. Variations in body weight or daily sodium excretion more accurately reflected sodium balance. But in any case, significant increase in sodium excretion could occur in the absence of change in filtration rate (patients 1 and 4).

(*2*) *Chlorides:* Plasma chloride levels typically were above normal in the patients with acute glomerulonephritis. The mean initial level for the 12 nephritic episodes was 114, $\sigma = 5.0$ meq./L, ranging up to 118 meq./L, as compared to 108, $\sigma = 3.3$ meq./L for 21 normal subjects observed under comparable conditions (*i.e.,* during concomitant measurement of filtration rate and renal plasma flow). All but one of the nephritic patients had plasma chloride levels exceeding 110 meq./L at some time, while only five of the 21 normal subjects had levels greater than this value and none exceeded 114 meq./L. With one exception, the plasma chloride level did not return toward normal within the period of observation as the patients improved from their acute nephritis. Chloride excretion was usually approximately the same as sodium.

TABLE VI

*Electrolytes in acute glomerulonephritis*

| | | | | SODIUM | | CHLORIDE | | POTASSIUM | | | INORGANIC PHOSPHATE | | | AMMONIA | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PATIENT NUMBER | DAYS AFTER ONSET | GLOMERULAR FILTRATION RATE (ml/min) | URINE FLOW (ml/min) | SERUM (meq/L) | EXCRETION RATE (μ/min) | PLASMA (meq/L) | EXCRETION RATE (μ/min) | PLASMA (meq/L) | EXCRETION RATE (μ/min) | PERCENT EXCRETION* | PLASMA (mg/100 ml) | EXCRETION RATE (mg/min) | PERCENT EXCRETION* | EXCRETION RATE (μ/min) | URINE pH |
| 1 | 8 | 82.6 | 1.87 | 136$^{E}$ | 40 | 112 | 40 | 4.9 | 27 | 6.6 | 3.8 | 0.50 | 15.7 | 44.0 | 5.2 |
| | 16 | 78.0 | 1.46 | 137 | 228$^{D}$ | 110 | 197 | 5.1 | 127 | 33.0 | 3.7 | 0.58 | 20.1 | 10.2 | 7.7 |
| | 30 | 106 | 1.26 | 137 | 337 | 112 | 262 | 4.7 | 89 | 18.4 | 3.1 | 0.42 | 12.7 | 13.3 | 5.0 |
| 2 | 7 | 100 | 6.09 | 137$^{E}$ | 550$^{D}$ | 117 | 562 | 4.7 | 64 | 13.8 | 2.7 | 0.29 | 10.5 | 46.4 | 6.3 |
| | 21 | 102 | 4.07 | 138 | 208 | 114 | 228 | 4.4 | 83 | 18.4 | 2.4 | 0.27 | 10.7 | 15.7 | 5.9 |
| 3 | 12 | 78.0 | 2.69 | 134$^{E}$ | 301 | 117 | 286 | 4.8 | 76 | 20.6 | 3.6 | 0.70 | 24.6 | 21.3 | 6.5 |
| | 22 | 73.4 | 1.72 | 138 | 215 | 114 | 233 | 4.7 | 70 | 20.5 | 2.7 | 0.58 | 29.9 | 34.8 | 5.7 |
| | 43 | 73.7 | 2.62 | 137 | 310 | 115 | 313 | 5.3 | 100 | 25.6 | 3.5 | 0.62 | 26.6 | 6.8 | 6.8 |
| 4 | 26 | 43.5 | 1.30 | 133$^{E}$ | 78 | 118 | 113 | 6.1 | 73 | 27.9 | 3.6 | 0.17 | 10.9 | 24.0 | 5.0 |
| | 30 | 43.6 | 1.75 | 137 | 209$^{D}$ | 114 | 134 | 5.7 | 136 | 55.0 | 3.9 | 0.32 | 18.9 | 6.2 | 5.7 |
| | 52 | 47.7 | 2.93 | 134 | 239 | 116 | 290 | 6.0 | 128 | 45.1 | 3.9 | 0.47 | 23.7 | 3.7 | 7.0 |
| 5 | 34 | 58.9 | 1.70 | 137$^{E}$ | 152$^{D}$ | 105 | 150 | 3.8 | 54 | 23.3 | 3.2 | 0.49 | 25.5 | 16.1 | 6.0 |
| | 43 | 64.8 | 6.33 | 132 | 153 | 106 | 278 | 4.2 | 43 | 15.6 | 3.6 | 0.45 | 19.7 | 27.7 | 5.7 |
| | 63 | 93.4 | 7.38 | 133 | 233 | 108 | 317 | 4.1 | 124 | 32.6 | 3.8 | 0.47 | 12.9 | 28.6 | 5.4 |
| | 118 | 94.0 | 3.77 | 137 | 213 | 104 | 250 | 4.2 | 101 | 25.3 | 3.4 | 1.21 | 37.9 | 17.0 | 4.7 |
| | 206 | 104 | 9.82 | 136 | 289 | 107 | 302 | 4.3 | 127 | 28.7 | 3.2 | 1.28 | 40.2 | 19.0 | 6.6 |
| 6 | 11 | 32.6 | 1.38 | 136$^{E}$ | 28 | 118 | 33 | 5.1 | 63 | 38.0 | 4.6 | 0.74 | 52.2 | 32.0 | 4.8 |
| | 20 | 41.4 | 2.27 | 138$^{E}$ | 192$^{D}$ | 115 | 147 | 4.6 | 93 | 51.6 | 4.5 | 0.95 | 50.9 | 4.7 | 6.7 |
| | 76 | 84.8 | 1.92 | 140 | 238 | 104 | 264 | 4.4 | 94 | 25.1 | 2.2 | 0.19 | 10.1 | 15.8 | 5.4 |
| 7 | 22 | 38.5 | 2.29 | 140$^{E}$ | 167 | 109 | 151 | 4.2 | 37 | 23.3 | 3.4 | 0.43 | 33.4 | 9.9 | --- |
| | 36 | 37.0 | 1.24 | 136 | 109$^{D}$ | 114 | 64 | 4.8 | 54 | 11.0 | 3.5 | 0.43 | 32.5 | 9.0 | 6.3 |
| | 54 | 47.7 | 2.21 | 135 | 98 | 113 | 133 | 4.5 | 87 | 40.9 | 3.4 | 0.53 | 32.3 | 7.2 | --- |
| 8, #1 | 12 | 54.9 | 1.93 | 134$^{E}$ | 253 | 114 | 213 | 6.2 | 79 | 23.3 | 4.0 | 0.49 | 22.5 | 4.6 | 7.6 |
| | 22 | 48.7 | 0.82 | 135 | 66$^{D}$ | 111 | 39 | 4.7 | 31 | 13.5 | 3.4 | 0.51 | 30.7 | 19.7 | --- |
| | 33 | 33.3 | 2.62 | 140 | 212$^{D}$ | 115 | 210 | 4.9 | 87 | 50.5 | 4.0 | 0.47 | 35.4 | 6.0 | 6.6 |
| | 48 | 35.8 | 2.96 | 137 | 260 | 116 | 255 | 6.3 | 131 | 57.7 | 4.3 | 0.85 | 55.1 | 2.4 | 7.4 |
| 8, #2 | 12 | 27.0 | 1.93 | 134$^{E}$ | 150 | 112 | 144 | 5.0 | 68 | 48.8 | 5.4 | 0.64 | 44.2 | --- | --- |
| | 27 | 41.4 | 1.88 | 132 | 246 | 116 | 272 | 5.4 | 96 | 42.6 | 4.4 | 0.80 | 43.4 | --- | --- |
| 9 | 17 | 61.7 | 0.62 | 137$^{E}$ | 49$^{D}$ | 112 | 44 | 4.7 | 46 | 16.0 | 3.5 | 0.26 | 12.0 | 11.5 | 5.2 |
| | 28 | 74.6 | 1.02 | 136 | 77 | 109 | 77 | 4.3 | 53 | 16.3 | 2.7 | 0.71 | 34.8 | 9.8 | 6.5 |
| 10 | 16 | 45.0 | 1.57 | 139$^{E}$ | 78$^{D}$ | 113 | 97 | 3.3 | 63 | 42.4 | 3.2 | 0.33 | 23.0 | 11.4 | 6.7 |
| | 35 | 38.6 | 1.26 | 139$^{E}$ | 76$^{D}$ | 112 | 76 | 3.8 | 41 | 28.2 | 3.6 | 0.42 | 30.5 | 9.9 | 6.2 |
| 11 | 15 | 21.9 | 0.96 | 140$^{E}$ | 27$^{D}$ | 118 | 42 | 7.3 | 53 | 32.4 | 3.9 | 0.33 | 40.1 | 6.2 | 5.4 |
| | 40 | 32.9 | 0.91 | 137$^{E}$ | 51$^{D}$ | 118 | 39 | 4.4 | 50 | 34.6 | 3.1 | 0.32 | 31.2 | 11.5 | 5.4 |
| | 67 | 54.4 | 3.18 | 139$^{E}$ | 278$^{D}$ | 120 | 272 | 5.5 | 89 | 29.6 | 3.0 | 0.49 | 30.6 | 6.8 | 7.5 |
| | 98 | 41.7 | 1.57 | 142 | 170 | 118 | 142 | 4.6 | 61 | 32.0 | 2.6 | 0.36 | 33.8 | 5.4 | 7.8 |
| | 117 | 55.5 | 1.57 | 138 | 131 | 118 | 182 | 5.3 | 92 | 31.4 | 2.4 | 0.34 | 26.4 | 4.0 | 6.1 |

Each datum represents the average of three consecutive periods.

E = During edema; D = During diuresis.

$\frac{\text{amount excreted}}{\text{amount filtered}} \times 100.$

μ = microequivalents.

(3) *Potassium:* The average initial plasma potassium level for the 12 acute episodes of nephritis was 5.0 meq./L, $\sigma = 1.08$, with a range of 3.3 to 7.3 meq./L. In contrast, the mean plasma potassium level, obtained under comparable circumstances (*i.e.*, during measurement of glomerular filtration rate and renal plasma flow) in 21 subjects without renal disease was 4.2 meq./L, $\sigma = 0.29$, with a range of 3.4 to 4.8. Only two of the normals had plasma potassium levels greater than 4.5 meq./L, while 10 of the 12 acute nephritics had values greater than this at one time or another. Patient 6 who had relatively low plasma potassium levels was the only one of the group who suffered vomiting prior to the observations. Abnormally high plasma potassium levels occurred in two patients whose filtration rates were not greatly reduced (patients 1 and 3), while conversely several of the patients with the lowest filtration rates had relatively normal plasma potassium levels. As was the case with plasma chloride, potassium plasma concentration was frequently above normal some days after recovery from acute nephritis.

The absolute values for potassium excretion in the urine showed a distinct tendency to rise as diuresis and recovery from the acute renal disease took place. Normally, less than 20 per cent of the potassium filtered at the glomeruli appears in the urine (18). However, the fraction of filtered potassium which was excreted exceeded this value at some time in 10 of the 12 acute episodes of glomerulonephritis. This phenomenon was most apparent among the patients with the greatest reductions in filtration rate but the relationship was not precise. Variations in excretion occurred independently of the plasma potassium level.

(4) *Inorganic phosphate:* The plasma level and urinary excretion of inorganic phosphate appear to be affected in acute glomerulonephritis to a lesser and more variable degree than potassium. The average initial plasma inorganic phosphate level for the 12 episodes was 3.7 mg./100 ml., $\sigma = 0.56$ mg./100 ml., as compared to 3.0 mg./100 ml., $\sigma = 0.47$ mg./100 ml. for 13 normal subjects under similar circumstances (*i.e.*, during measurement of filtration rate). Only three of the nephritics had at any time a plasma inorganic phosphate level greater than the mean normal plus two standard deviations. Plasma levels of potassium and inorganic phosphate did not necessarily parallel one another. Unlike potassium, urinary inorganic phosphate excretion increased with improvement in the renal lesion only occasionally. The fraction of filtered inorganic phosphate which was excreted by 13 normal subjects was quite variable, with a range from 2.3 to 26.5 mg./100 ml., a mean of 13.0 mg./100 ml., $\sigma = 7.5$. Nevertheless, the mean normal value plus two standard deviations was exceeded in nine of the 12 episodes of acute nephritis.

(5) *Ammonia excretion:* The mean ammonia excretion rate in nine normal subjects, measured during the estimation of glomerular filtration rate and renal plasma flow, was 13 microeq./minute. One subject's rate was 2.7 microeq./minute, while the rates in the remainder varied between 11.6 and 18.2 microeq./minute. Somewhat increased rates of ammonia excretion, varying between 21.3 and 46.4 microeq./minute, were observed in five patients (patients 1–4 and 6) during the early phases of their acute glomerulonephritis (within two weeks of onset). The excretion rates in these patients subsequently fell to between 3.7 and 15.8 microeq./minute. These changes in ammonia excretion could not be correlated precisely with the urinary pH. Several of the remaining patients occasionally exhibited ammonia excretion greater than the normal group, but generally the rates were in the neighborhood of 10 microeq./minute or less.

## DISCUSSION

The pattern of renal dysfunction in acute glomerulonephritis appears to be quite characteristic. Typically the glomerular filtration rate is reduced. Function of the proximal convoluted tubules as measured by the maximum excretory capacity for diodrast ($Tm_D$) or p-aminohippurate ($Tm_{PAH}$) may also be reduced, but low filtration rate to Tm ratios reflect the greater impairment of glomerular activity. Reabsorptive capacity for glucose may also be impaired but this function and the excretory capacity of the tubules may be affected to different degrees in the same patient.

That the reduced filtration fraction observed in acute glomerulonephritis represents a greater barrier to glomerular filtration than to the passage of blood through the glomerular apparatus has been suggested in an earlier study (1). It is recognized that, in fatal instances the glomerular tuft changes may be such as to obstruct the flow of blood. This mechanism may account for the usual autopsy description of "bloodless" glomeruli in such patients.[4] However, in milder forms of the disease, high RPF/Tm ratios indicate relative renal hyperemia, and at times, actual renal hyperemia (1).

The characteristic renal functional disturbances of acute glomerulonephritis can persist in spite of circumstances that are ordinarily associated with quite different patterns of dysfunction. Thus, congestive heart failure (19–21) and hypertension (22) are characterized by considerable reduction in the renal plasma flow and by increased filtration fraction. However, a decreased filtration fraction was observed in one patient whose acute glomerulonephritis was complicated by congestive heart failure, while the other patient with the same complication had a normal filtration fraction. Similarly, low filtration fractions occurred in many of the patients with acute nephritis in spite of significant hypertension.

The majority of instances of acute glomerulonephritis heal completely, especially in the younger age group. Unfortunately, accurate prognosis in the early phases of the disease is practically impossible. Renal function studies during the early stages of acute nephritis are of little or no assistance in determining the prognosis. Although the patients in this study who had the greatest impairment of function did not recover, many observers have commented that even the most severe uremia is not incompatible with complete recovery (7). Conversely, the acute disease may be so mild as to almost escape attention and yet fail to heal (7).

The functional changes during the exacerbation of chronic glomerulonephritis are similar to those of the initial acute episode. However, because renal function is usually already reduced to some degree in patients with the chronic disease, recovery of function following the exacerbation is not ordinarily as complete as it is following the initial acute attack.

Edema is one of the most common manifestations of acute glomerulonephritis. Many observers believe that generalized capillary damage accounts for the edema in the majority of instances. This belief is based chiefly on the common localization of edema about the face and especially about the eyes (23), on the occurrence of edema and hypertension prior to or even without urinary abnormalities (24) and on older observations that edema fluid of acute glomerulonephritis contained relatively high amounts of protein (25). However, obvious accumulation of edema in the loose tissue of the face is not surprising in patients with fluid retention who are confined to bed and who do not have local or general elevation of venous pressure. Nor does the development of edema and hypertension prior to the appearance of urinary abnormalities preclude the possibility of early renal functional involvement. And the most recent observation on the edema fluids of acute nephritis reports the low protein content characteristic of transudates rather than of inflammatory reactions (26).

Congestive heart failure (27) was a contributing factor to the edema of only two of the 12 patients with acute glomerulonephritis in this study while none exhibited the nephrotic syndrome. A renal mechanism for fluid and salt retention in acute nephritis, therefore, should receive serious consideration. The observed reduction of filtration rate may well play some role in the pathogenesis of the edema of acute glomerulonephritis. This is especially likely in those patients whose filtration rates were reduced suddenly and severely. However, at least one patient with massive edema had a filtration rate within the normal range. Further, the urea clearance in acute glomerulonephritis is not infrequently reduced out of proportion to the filtration rate and the degree of oliguria. Reabsorption of urea by the renal tubules appears to represent passive diffusion (28). A possible explanation of the low urea/inulin clearance ratio in these instances of acute glomerulonephritis could be damage to the renal epithelium. Such damage perhaps could permit increased back diffusion of salt as well as of urea. Certainly, the present data indicate, at least, that the diuresis during recovery from an episode of acute glomerulonephritis can take place without a concomitant increase in glomerular filtration rate. The increases in urea clearance with diuresis were not entirely dependent on the increase in urine flow, and thus may have been the result of reconstitution of the renal epithelium with return of function toward normal.

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[4] The amount of blood in the glomerular capillaries in an autopsy specimen does not necessarily reflect the amount of blood present during life. Very few erythrocytes are seen in the glomerular capillaries of animals sacrificed with entirely normal kidneys.

Abnormalities in the plasma concentration and excretion of sodium, potassium, chloride and inorganic phosphate, although common in acute glomerulonephritis were not well correlated with observed changes in renal functions. An increased fraction of filtered potassium excreted in the urine was one of the most consistent electrolyte abnormalities in acute nephritis. This phenomenon has previously been noted in patients with advanced renal failure (29) and probably represents a fortunate shift in glomerulo-tubular balance that permits maintenance of the plasma potassium level as closely as possible to the normal range in the face of decreased filtered loads of the ion. Whether decreased tubular absorption of potassium or increased tubular excretion, both of which theoretically can occur in chronic renal disease (29, 30), is responsible is not certain.

Ammonia excretion appeared to be increased somewhat during the early phases of acute glomerulonephritis. Although rates of ammonia excretion did not correlate precisely with urinary pH, the increased ammonia in the urine perhaps represented a response to the acidosis that may have been present. Subsequently, ammonia excretion fell below the rates usually observed in normals, perhaps because of damage to the distal renal tubule cells.

## SUMMARY AND CONCLUSIONS

1. The typical renal functional disturbances in an acute episode of glomerulonephritis issues from the characteristic glomerular lesion which leads to an excessive reduction in filtration rate and filtration fraction. Excretory and reabsorptive functions, as measured by $Tm_{PAH}$ and Tm glucose, respectively, are less extensively reduced.

2. The changes in renal functions are similar in the exacerbation in chronic glomerulonephritis.

3. The typical changes are noted in spite of hypertension or the development of congestive heart failure, conditions otherwise characterized by an increased filtration fraction.

4. The degree of abnormality and altered interrelations of renal functions are of little assistance in arriving at a prognosis. Considerable improvement in all functions usually follows an acute episode, although the rate of recovery is highly variable. Function also improves following an exacerbation but does not return to normal because of pre-existing impairment effected by chronic disease.

5. Salt and water diuresis during the recovery phase of acute glomerulonephritis may take place without change in glomerular filtration rate. Urea clearance is sometimes reduced out of proportion to the filtration rate in acute glomerulonephritis, and during recovery may increase without change in filtration rate.

6. Serum sodium concentration is normal or slightly low while plasma chloride, potassium and inorganic phosphate levels are sometimes above normal. These changes are not related to the degree of abnormality observed in renal functions.

7. Sodium and chloride excretion more or less parallel one another. Potassium excretion is depressed during the early stages of acute glomerulonephritis and increases during the diuresis of the recovery phase. A greater fraction than normal of filtered potassium and inorganic phosphate appear in the urine in the majority of patients.

8. Ammonia excretion is greater than normal during the early phases of acute glomerulonephritis but subsequently falls below the usual normal range.

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# THE COLLAPSE PRODUCED BY VENOUS CONGESTION OF THE EXTREMITIES OR BY VENESECTION FOLLOWING CERTAIN HYPOTENSIVE AGENTS[1]

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(Submitted for publication December 14, 1950; accepted, February 28, 1951)

It is well recognized that interruption of the lower thoracic and lumbar sympathetic nerves, or the administration of certain hypotensive drugs particularly sodium nitrite and the "sympatholytic" agents may cause hypotension and collapse in the erect position. It has been postulated that this hypotensive reaction is due to pooling of blood in the dependent parts of the body, particularly in the splanchnic vascular bed with consequent failure of venous return and, hence, of cardiac output (1).

During studies on the hemodynamic effects of hypotensive drugs in man we have observed sudden and marked reductions of arterial pressure frequently with collapse occurring in the supine position. This phenomenon was induced by subjecting the limbs to brief periods of venous congestion, after the administration of certain hypotensive agents (2). This paper presents data on the types of agents that cause this phenomenon as well as an analysis of the mechanisms whereby the effect is produced.

## MATERIALS AND METHODS

The subjects were patients both hypertensive and normotensive from the hospital wards. Arterial pressure was recorded either by the usual auscultatory method or optically with a Hamilton manometer connected to an 18 gauge needle inserted into the brachial artery.

With the patient in the supine position, inflatable cuffs connected to a large air reservoir bottle were applied proximally to both thighs and one arm and were held in place by covering the cuffs and adjacent skin with gauze bandage. In all of the hypertensive patients the limbs were congested with a pressure of 100 mm. Hg and in the normotensive subjects at pressures of 60 to 80 mm. Hg. Before and after the administration of each of the various drugs tested the extremities were congested for five minutes unless collapse supervened. After each drug the blood pressure was recorded frequently. The point at which the blood pressure leveled off or began to rise was taken as the point for applying venous congestion. A similar procedure was used in the venesection experiments.

The volume of blood trapped in the congested extremities was determined according to the method of Ebert and Stead (3) following a congestion period of five minutes, instead of seven to 10 minutes as used by those investigators. The shorter congestion period may account for the fact that in the present experiments the volume of blood trapped in the extremities usually was smaller than that observed by Ebert and Stead.

Following the congesting period the pressure in the cuffs was raised to 250 mm. Hg after which the dye T-1824 was injected. Samples were drawn at eight and 10 minutes afterwards. The occluding cuffs were then released and further sampling was carried out at 18 and 20 minutes. The latter samples were used to determine total blood volume while the former were used to determine the volume of that portion of the body not removed from the general circulation by the occluding cuffs. It was found that the dye density values of the eight and 10 minute samples agreed closely with each other indicating that mixing was essentially complete at the end of eight minutes. Similarly the 18 minute were in good agreement with the 20 minute samples. The results of other investigations (4–7) also indicate that in normal subjects equilibration of dye is complete in less than eight minutes. Hence, it seems probable that this method was sufficiently accurate for the present purposes.

The segment plethysmograph used to determine changes in calf volume was the same as that described by Wilkins and Eichna (8) with the exception that proximal and distal blood pressure cuffs were not applied since in the present experiments the plethysmograph was used to determine volume changes rather than blood flow.

In the venesection experiments a 15 gauge needle was inserted into an antecubital vein and the blood introduced

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[1] Supported in part by research grants from the National Heart Institute, U. S. Public Health Service, E. R. Squibb and Sons, New York, N. Y., and Irwin, Neisler and Company, Decatur, Ill.

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into a vacuum flask containing 130 cc. of acid citrate dextrose solution (ACD). Immediately following completion of the bleeding period the flask was inverted, and then pressurized by raising it and introducing air into it in order to hasten the return of blood to the circulation.

## RESULTS

### *Effect of various hypotensive agents in the production of collapse during venous congestion of the extremities*

In the control period, prior to the administration of any drug only two of the 24 subjects exhibited a significant reduction of arterial pressure during congestion of the extremities for a five minute period. In these two cases (G. S. and J. L., Table I) the reductions in average $\left(\frac{\text{systolic} + \text{diastolic}}{2}\right)$ arterial pressure were 10 and 12 per cent, respectively. Neither of these patients exhibited signs of collapse.

Five patients who received sodium nitrite in doses of 90 to 210 mg. by mouth exhibited changes in average arterial pressure varying from + 1 to − 44 per cent (mean − 25 per cent) during congestion of the extremities. Three of these subjects developed marked hypotension and symptoms of collapse within two to three minutes after the congesting pressures were applied.

Five subjects were given 0.5 mg. of dihydroergocornine (DHO) intravenously. During congestion the changes in average arterial pressure in these patients varied between + 6 and − 48 per cent (mean − 27 per cent). Three of these subjects developed signs of collapse prior to the end of the five minute congesting period.

In three subjects given tetraethylammonium (250 to 400 mg. intravenously) none developed collapse during congestion of the limbs although all exhibited reductions in average arterial pressure varying from − 4 to − 22 per cent (mean − 15 per cent). However, all of the four subjects given the longer acting and more potent ganglionic blocking agent hexamethonium ($C_6$) developed collapse within two to five minutes of venous congestion. The reduction of arterial pressure in this group of patients varied between − 16 and − 56 per cent (mean − 37 per cent).

TABLE I

*The effect of various hypotensive agents on the arterial pressure during congestion of the extremities of unsympathectomized patients*

| Pt. | Sex | Age | Drug | Dose and route | Control | | | Post-drug period | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Arterial pressure | | Change mean* arterial pressure | Arterial pressure | | Change mean arterial pressure | Collapse |
| | | | | | Basal | After five min. of congestion | | Basal | After five min. or less of congestion | | |
| | | | | | *mm. Hg* | *mm. Hg* | *per cent* | *mm. Hg* | *mm. Hg* | *per cent* | |
| R. M. | M | 24 | Sodium nitrite | 180 mg. P.O. | 200/120 | 190/112 | − 5 | 180/106 | 155/100 | −11 | 0 |
| A. A. | M | 48 | Sodium nitrite | 120 mg. P.O. | 226/128 | 246/146 | +11 | 170/115 | 98/66† | −44 | + |
| E. C. | F | 43 | Sodium nitrite | 90 mg. P.O. | 225/109 | 210/118 | − 2 | 178/99 | 88/70‡ | −43 | + |
| M. S. | M | 22 | Sodium nitrite | 180 mg. P.O. | 118/68 | 114/64 | − 4 | 96/50 | 68/40 | −26 | + |
| A. P. | M | 28 | Sodium nitrite | 210 mg. P.O. | 180/110 | 174/110 | − 2 | 170/106 | 176/106 | + 1 | 0 |
| K. M. | F | 51 | DHO | 0.5 mg. I.V. | 218/120 | 214/128 | + 1 | 184/94 | 90/56 | −48 | + |
| J. S. | F | 45 | DHO | 0.5 mg. I.V. | 194/110 | 192/102 | − 3 | 174/90 | 176/99 | + 6 | 0 |
| E. M. | F | 29 | DHO | 0.5 mg. I.V. | 204/104 | 191/112 | + 3 | 188/104 | 108/56 | −43 | + |
| J. G. | M | 28 | DHO | 0.5 mg. I.V. | 126/84 | 111/79 | − 9 | 130/82 | 95/65 | −25 | 0 |
| S. K. | M | 43 | DHO | 0.5 mg. I.V. | 120/82 | 114/78 | − 5 | 114/76 | 85/62 | −23 | + |
| R. K. | M | 49 | TEAC | 300 mg. I.V. | 197/120 | 212/133 | + 9 | 160/106 | 127/89 | −19 | 0 |
| E. S. | F | 52 | TEAC | 250 mg. I.V. | 255/142 | 252/149 | + 1 | 236/146 | 220/142 | − 4 | 0 |
| J. L. | M | 28 | TEAC | 400 mg. I.V. | 140/80 | 118/78 | −12 | 128/80 | 100/62 | −22 | 0 |
| G. S. | M | 42 | $C_6$ | 50 mg. I.V. | 180/110 | 160/100 | −10 | 160/100 | 80/60 | −56 | + |
| A. J. | F | 49 | $C_6$ | 50 mg. I.V. | 170/120 | 164/126 | 0 | 128/100 | 90/80 | −25 | + |
| T. B. | M | 40 | $C_6$ | 50 mg. I.V. | 170/110 | 155/120 | − 2 | 110/80 | 85/75 | −16 | + |
| L. M. | M | 37 | $C_6$ | 50 mg. I.V. | 170/115 | 160/120 | − 1 | 150/110 | 75/155 | −50 | + |
| S. W. | M | 54 | Sodium amytal | 0.25 gm. I.V. | 231/118 | 220/123 | − 2 | 200/110 | 192/110 | − 6 | 0 |
| A. L. | M | 27 | Sodium amytal | 0.75 gm. I.V. | 130/96 | 122/92 | − 5 | 128/100 | 118/100 | − 5 | 0 |
| E. S. | M | 61 | Sodium amytal | 0.5 gm. I.V. | 180/104 | 176/108 | 0 | 138/100 | 124/96 | − 8 | 0 |
| B. G. | M | 38 | Veratrone | 0.8 cc. I.M. | 210/128 | 204/124 | − 3 | 164/96 | 162/100 | + 1 | 0 |
| C. B. | F | 56 | Veratrone | 1.0 cc. I.M. | 272/180 | 276/182 | + 1 | 142/94 | 146/100 | + 4 | 0 |
| J. Mc. | M | 52 | Vertavis | 40 CRAW V.PO. | 265/160 | 266/165 | + 1 | 155/117 | 145/131 | + 2 | 0 |
| E. S. | F | 52 | Vertavis | 40 CRAW V.PO. | 255/142 | 252/149 | + 1 | 200/112 | 196/114 | − 1 | 0 |

* "Mean" arterial pressure $= \frac{\text{systolic} + \text{diastolic pressure}}{2}$.

† Collapse occurred $2\frac{3}{4}$ minutes after application of congesting pressure.

‡ Collapse occurred two minutes after application of congesting pressure.

TABLE II

*The amount of blood trapped in the extremities subjected to venous congestion before and after sodium nitrite, dihydroergocornine and hexamethonium*

| Subject | Control | | | After drug | | | |
|---|---|---|---|---|---|---|---|
| | Volume of blood in trunk, head and arm | Total blood volume | Blood trapped in congested limbs | Drug dose and route | Volume of blood in trunk, head and arm | Total blood volume | Blood trapped in congested limbs |
| | *cc.* | *cc.* | *cc.* | | *cc.* | *cc.* | *cc.* |
| J. G.* | 4,700 | 6,350 | 1,650 | DHO<br>0.5 mg. I.V. | 4,860 | 6,400 | 1,540 |
| S. K.* | 4,150 | 5,480 | 1,330 | DHO<br>0.5 mg. I.V. | 4,070 | 5,460 | 1,390 |
| M. S.* | 4,040 | 5,075 | 1,025 | Sodium nitrite<br>210 mg. P.O. | 4,055 | 5,020 | 965 |
| R. M.* | 2,990 | 4,230 | 1,240 | Sodium nitrite<br>180 mg. P.O. | 3,010 | 4,280 | 1,270 |
| I. P. | 5,540 | 6,660 | 1,120 | Hexamethonium<br>50 mg. I.V. | 5,450 | 6,540 | 1,090 |

* Data on the changes in arterial pressure given in Table I.

No significant reductions of arterial pressure occurred during congestion of the extremities of three patients given 0.25 to 0.75 gm. of sodium amytal intravenously, two subjects given 0.8 or 1.0 cc. of Veratrone (tincture of veratrum viride) intramuscularly and two patients given 40 Craw units of Vertavis (whole powdered root and rhizomes of veratrum viride) orally. In all of the latter cases significant reductions of basal arterial pressure had occurred following the administration of veratrum viride.

The signs and symptoms related to the collapse state induced by sodium nitrite and the "sympatholytic" agents during congestion of the extremities resembled those observed in other types of acute hypotension such as carotid sinus and vaso-vagal syncope, or postural hypotension. The pulse pressure became small and bradycardia frequently developed. Pallor, yawning, sweating and vomiting sometimes occurred. The patients experienced faintness, giddiness and at times nausea.

For example, patient E. C. (Table I) who received 180 mg. of sodium nitrite by mouth 30 minutes prior to congestion exhibited a marked reduction of arterial pressure from 178/100 to 88/70 mm. Hg within three minutes after the venous tourniquets had been applied. Accompanying the hypotensive response there was profuse sweating, pallor and bradycardia. The patient complained of faintness and nausea. Release of the congesting pressure at this time and tilting the patient into a five degree head-down position failed to relieve her symptoms. The arterial pressure continued to fall and one minute after releasing the pressure in the cuffs it was 48/33 mm. Hg and the heart rate was 58 per minute. Four minutes after releasing the congesting pressure the arterial pressure had risen to 110/78 mm. Hg and the heart rate to 72 per minute at which time the patient felt improved.

### *The amount of blood trapped in the congested limbs*

In one hypertensive and four normotensive subjects the amount of blood trapped in the congested limbs was determined by the dye method of Ebert and Stead (3). In the control period the amount of blood present in both lower extremities and one upper extremity during congestion varied between 1,025 and 1,650 cc. with a mean of 1,273 cc. (Table II, Figure 1). There were no significant changes in arterial pressure. Two subjects received sodium nitrite, two were given DHO and one received hexamethonium. During the congestion period following these drugs all of the subjects exhibited significant reductions of arterial pressure and three developed marked hypotension and symptoms of collapse (Table II). The latter occurred two to

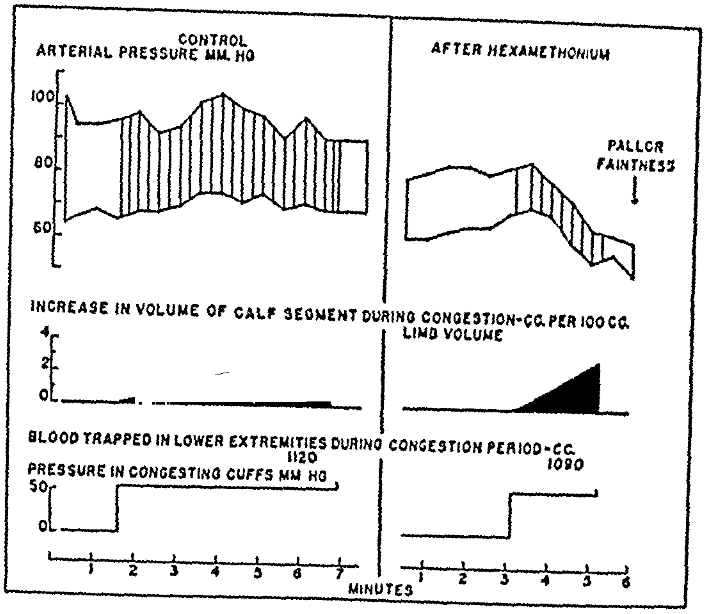

FIG. 1. CHART OF ARTERIAL PRESSURE, INCREASE IN VOLUME OF CALF SEGMENT, AMOUNT OF BLOOD TRAPPED IN THE EXTREMITIES AND CONGESTING CUFF PRESSURES BEFORE AND AFTER INTRAVENOUS HEXAMETHONIUM (50 mg.) IN SUBJECT I.P., NORMAL MALE, AGED 28

In the control period the arterial pressure was well maintained during venous congestion of the extremities for 5¼ minutes. Following hexamethonium and 2¼ minutes after the venous tourniquets had been applied, hypotension and collapse developed. However, the amount of blood trapped in the congested extremities was less and the increase in volume of the calf segment was not as great as in the control period.

four minutes after the beginning of congestion. This necessitated application of occluding pressure prior to the end of the five minute congestion period. This was done in order to prevent blood from either entering or leaving the limbs while the patient was tilted into a head-down position and allowed to recover during the time that the blood volume determinations were being completed.

The amount of blood present in the congested limbs during the experimental period varied between 965 and 1,540 cc. (mean 1,263 cc.). In two subjects there was a slight increase in the amount of blood trapped in the limbs in the post-drug as compared to the pre-drug congestion period and in three cases a slight decrease. In no case did the pre-and post-drug values vary from each other by more than 12 per cent.

### *Changes in calf volume during venous congestion of the extremities*

In order to corroborate these observations indicating that no more blood is trapped in the limbs during venous congestion after as compared with before the administration of agents which induce "congestion collapse," the volume changes in the calf were measured using a plethysmograph. Hexamethonium was administered to all of the subjects.

In the control period the increase in volume of the calf segment during four and one-half to six and one-half minutes of venous congestion varied between 2.4 to 5.2 cc. per 100 cc. of limb volume (mean 3.8 cc.) (Table III, Figure 1). Hypotension did not occur in any of these subjects.

After hexamethonium five of the six subjects developed severe hypotension during periods of congestion of one and one-half to four minutes. The increase in calf volume varied between 2.0 and 3.6 cc. (mean 2.7 cc.). Subject P. G. did not develop severe hypotension during application of venous tourniquets for five and one-half minutes. The *rate of increase* in calf volume during congestion usually was greater after as compared to before hexamethonium (Figure 1). However, in

TABLE III

*The increase in volume of the calf segment during application of venous tourniquets before and after the administration of hexamethonium*

| Subject | Control | | | After hexamethonium (50 mg. I.V.) | | |
|---|---|---|---|---|---|---|
| | Increase in volume of calf segment | Duration of venous congestion | Development of hypotension | Increase in volume of calf segment | Duration of venous congestion | Development of hypotension |
| | *cc. per 100 cc. limb volume* | *minutes* | | *cc. per 100 cc. limb volume* | *minutes* | |
| A. G. | 3.0 | 5¾ | 0 | 2.8 | 1¾ | + |
| I. P. | 4.0 | 5¼ | 0 | 3.0 | 2¼ | + |
| A. H. | 5.2 | 6¼ | 0 | 2.3 | 2½ | + |
| P. G. | 2.8 | 4½ | 0 | 5.0 | 5½ | 0 |
| J. D. | 3.0 | 5 | 0 | 3.6 | 4 | + |
| L. B. | 2.4 | 6½ | 0 | 2.0 | 1½ | + |

TABLE IV

*The effect of dihydroergocornine (DHO), hexamethonium ($C_6$), and sodium nitrite on the arterial pressure and heart rate following venesection and subsequent return of the removed blood to the circulation*

| Pt. | Drug, dose and route | Before drug | | After drug and before venesection | | During venesection | | | | During return of removed blood | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Arterial pressure | Heart rate | Arterial pressure | Heart rate | Blood removed | Time from beginning of venesection | *Arterial pressure* | *Heart rate* | Blood returned | Time from beginning of return | *Arterial pressure* | *Heart rate* |
| | | *mm. Hg* | *per min.* | *mm. Hg* | *per min.* | *cc.* | *minutes* | *mm. Hg* | *per min.* | *cc.* | *minutes* | *mm. Hg* | *per min.* |
| L. H. | $C_6$<br>50 mg.<br>I.V. | 170/100 | 82 | 140/80 | 86 | 100 | 2 | 128/80 | 92 | 100 | 2 | 130/70 | 81 |
| | | | | | | 200 | 3 | 120/75 | 92 | 200 | 8 | 140/80 | 80 |
| | | | | | | 300 | 4 | 115/75 | 100 | 300 | 16 | 150/85 | 80 |
| | | | | | | 400 | 8 | 105/70 | 100 | 400 | 21 | 172/94 | 80 |
| | | | | | | 500 | 12 | 98/60 | 80 | 500 | 26 | 180/96 | 82 |
| J. B. | $C_6$<br>50 mg.<br>I.V. | 220/120 | 88 | 140/90 | 84 | 100 | 1 | 120/80 | 84 | 100 | 1 | 90/65 | 84 |
| | | | | | | 200 | 2 | 120/85 | 80 | 200 | 2 | 100/70 | 80 |
| | | | | | | 300 | 5 | 115/75 | 80 | 350 | 4 | 130/90 | 80 |
| | | | | | | 400 | 6 | 98/70 | 88 | 450 | 7 | 135/95 | 76 |
| | | | | | | 500 | 8 | 80/65* | 80 | 500 | 9 | 140/100 | 76 |
| M. D. | DHO<br>0.5 mg.<br>I.V. | 130/100 | 84 | 130/100 | 84 | 100 | 2 | 115/85 | 80 | 100 | 3 | 110/80 | 88 |
| | | | | | | 200 | 4 | 110/85 | 92 | 200 | 6 | 105/80 | 84 |
| | | | | | | 300 | 7 | 108/82 | 96 | 300 | 7 | 100/85 | 80 |
| | | | | | | 400 | 12 | 100/80 | 96 | 400 | 9 | 110/85 | 80 |
| | | | | | | 500 | 15 | 96/72 | 104 | 500 | 12 | 110/90 | 72 |
| M. C. | DHO<br>0.5 mg.<br>I.V. | 220/160 | 80 | 220/140 | 80 | 100 | 2 | 190/120 | 80 | 125 | 4 | 160/110 | 76 |
| | | | | | | 200 | 4 | 140/105 | 76 | 250 | 9 | 180/120 | 76 |
| | | | | | | 250 | 5 | 120/90* | 80 | | | | |
| E. A. | $NaNO_2$<br>210 mg.<br>P.O. | 122/82 | 94 | 122/82 | 96 | 100 | 2 | 108/84 | | | | | |
| | | | | | | 200 | 5 | 110/82 | | | | | |
| | | | | | | 300 | 9 | 108/78 | | | | | |
| | | | | | | 400 | 17 | 105/72 | | | | | |
| E. R. | $NaNO_2$<br>240 mg.<br>P.O. | 240/140 | 60 | 180/120 | 84 | 100 | 1 | 180/120 | 92 | 120 | 2 | 135/95 | 80 |
| | | | | | | 200 | 4 | 150/100 | 92 | 200 | 3 | 145/100 | 76 |
| | | | | | | 300 | 7 | 150/110 | 96 | 300 | 7 | 145/110 | 80 |
| | | | | | | 400 | 10 | 160/100 | 104 | 400 | 11 | 160/120 | 72 |
| | | | | | | 525 | 15 | 125/85 | 84 | 525 | 15 | 160/120 | 72 |
| W. O. | $NaNO_2$<br>120 mg.<br>P.O. | 190/115 | 80 | 195/112 | 80 | 150 | 1 | 165/110 | 84 | 150 | 2 | 160/108 | 84 |
| | | | | | | 300 | 2 | 155/110 | 88 | 400 | 4 | 150/110 | 80 |
| | | | | | | 400 | 4 | 155/110 | 88 | 500 | 6 | 160/110 | 82 |
| | | | | | | 500 | 6 | 155/110 | 88 | | | | |

* Yawning, pallor, feels faint.

four of the five patients who developed hypotension during the post-drug congestion period marked reduction in arterial pressure occurred before the *total* increase in calf volume was as great as that observed at the end of the pre-drug congestion period.

The estimation of the amount of blood trapped in the congested limbs as determined by the plethysmographic method agrees approximately with that obtained by the dye method. Previous studies estimate that about 750 cc. of blood are normally present in the lower extremities (3). The average amount of blood found in the congested extremities was 1,270 cc. Thus, the excess of blood pooled out was approximately 1,270 − 750 or 520 cc. Using the plethysmographic method the average increase in calf volume during the control period was approximately 5.6 cc. per 100 cc. of limb volume. If the volume of both lower extremities is estimated to be approximately 10 liters the amount of blood pooled out would be 5.6 × 100 or 560 cc. It should be emphasized that this is a crude estimation since the plethysmograph measures the volume change in only one segment of the limb which may represent a selective rather than a representative portion of the vascular bed being congested.

### *Effects of certain hypotensive drugs on the arterial pressure during venesection*

Seven patients, of whom five were hypertensive, were bled rapidly from an antecubital vein with removal of 250 to 525 cc. of blood (Table IV). Two patients received 0.5 mg. of dihydroergocornine intravenously, two were given 50 mg. of hexamethonium by the same route and three received 120 to 240 mg. of sodium nitrite orally. All of these patients exhibited reductions of average arterial pressure varying from − 10 to − 42 per cent; in five patients the reduction exceeded − 24 per cent. Two patients developed signs of collapse after withdrawal of 250 and 500 cc. of blood, respectively.

The arterial pressure did not fall precipitously at any point in the bleeding period. Instead there usually was a stepwise decrease with each 100 cc. of blood removed (Table IV and Figure 2). Similarly on returning the blood to the circulation the increase in arterial pressure paralleled the rate at which the blood was reinfused. Thus, there was no evidence of a critical amount of blood loss as in untreated subjects beyond which "compensation" fails and the arterial pressure falls suddenly.

As a control the arterial pressures of eight blood donors were measured during venesection. No pretreatment with hypotensive agents had been given. The removal of 500 cc. of blood at a similar rate from each of the donors resulted in a decrease of average arterial pressure of − 8 to + 2 per cent (average − 3 per cent).

### *Absence of congestion collapse after hypotensive drugs in partially sympathectomized subjects*

Eight hypertensive patients who had undergone either a lumbodorsal, or a transthoracic splanchnicectomy four months to five years previously were studied. Two patients received sodium nitrite in doses of 150 to 180 mg. orally, one received 0.3 mg. of the mixed dihydrogenated alkaloids of ergot (CCK) intravenously and five were given 50 mg. of $C_6$ intravenously (Table V). In only two subjects were significant decreases in average arterial pressure observed (− 11 and − 22 per cent, respectively) during the post drug con-

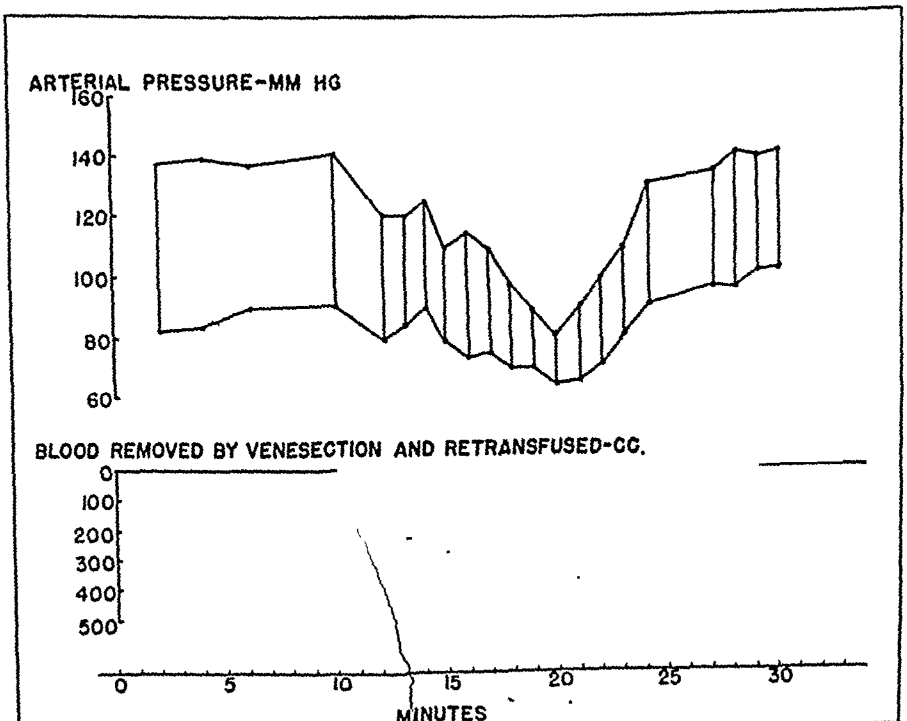

FIG. 2. CHART OF ARTERIAL PRESSURE AND THE AMOUNT OF BLOOD REMOVED BY VENESECTION AND RETRANSFUSED IN PATIENT J. B., FEMALE, AGED 48

Hexamethonium (50 mg.) was given intravenously 30 minutes prior to beginning the venesection. Yawning, pallor and a feeling of faintness developed when the blood pressure fell to its lowest point.

TABLE V

*The effect of sodium nitrite, $C_6$ and CCK on the arterial pressure during congestion of the extremities of sympathectomized patients*

| Pt. | Sex | Age | Drug | Dose and route | Control: Arterial pressure: Basal | Control: Arterial pressure: After five min. of congestion | Control: Change mean* arterial pressure | Post-drug period: Arterial pressure: Basal | Post-drug period: Arterial pressure: After five min. of congestion | Post-drug period: Change mean arterial pressure | Collapse |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | *mm. Hg* | *mm. Hg* | *per cent* | *mm. Hg* | *mm. Hg* | *per cent* | |
| M. C. | F | 44 | Sodium nitrite | 150 mg. P.O. | 164/100 | 159/104 | 0 | 156/101 | 160/105 | + 3 | 0 |
| P. D. | M | 38 | Sodium nitrite | 180 mg. P.O. | 170/110 | 168/108 | −2 | 146/94 | 134/90 | − 7 | 0 |
| H. F. | M | 36 | CCK | 0.3 mg. I.V. | 212/128 | 215/135 | +3 | 225/135 | 220/140 | 0 | 0 |
| H. M. | F | 43 | $C_6$ | 50 mg. I.V. | 210/125 | 200/130 | −1 | 150/90 | 160/95 | + 6 | 0 |
| M. D. | F | 44 | $C_6$ | 50 mg. I.V. | 190/120 | 185/120 | −1 | 140/100 | 130/100 | − 4 | 0 |
| D. H. | F | 35 | $C_6$ | 50 mg. I.V. | 220/140 | 205/140 | −5 | 140/120 | 140/120 | 0 | 0 |
| C. M. | M | 47 | $C_6$ | 50 mg. I.V. | 210/140 | 210/138 | −1 | 170/125 | 140/120 | −11 | 0 |
| P. D. | M | 38 | $C_6$ | 50 mg. I.V. | 164/110 | 160/108 | −2 | 124/95 | 90/60 | −22 | 0 |

* "Mean" arterial pressure $= \frac{\text{systolic + diastolic pressure}}{2}$.

gestion period. Neither of these subjects developed signs or symptoms of collapse. Six of the eight had significant reductions of basal arterial pressure following the hypotensive agents and all of these exhibited increased postural hypotension.

## DISCUSSION

The development of collapse during venous congestion of the extremities has been observed in normal subjects (3) and in patients who have suffered recent blood loss (9). Our observations indicated, however, that patients given certain hypotensive agents are unusually susceptible to the development of collapse produced by venous tourniquets and/or hemorrhage. The type of hypotensive agents producing this phenomenon were those which also cause postural hypotension (10, 11), that is, sodium nitrite, hexamethonium, tetraethylammonium and the dihydrogenated alkaloids of ergot; whereas hypotensive drugs such as veratrum viride and sodium amytal which do not ordinarily cause postural hypotension also did not induce congestion collapse.

The increased susceptibility to collapse during venous congestion following sodium nitrite and the sympatholytic agents conceivably might be due to one of several mechanisms. Ebert and Stead have demonstrated that considerable amounts of blood may be trapped out of the central circulation during venous congestion of the limbs (3). They showed that if venous tourniquets were applied for ten minutes or longer almost 30 per cent of the total blood volume may be present in the congested extremities resulting in collapse even in normal untreated subjects. It should be noted that the period of venous congestion had to be longer than five minutes to produce collapse in these untreated subjects. Since, in the present experiments with sodium nitrite and the "sympatholytic agents" hypotension appeared early in the congestion period it seemed possible that these agents induced greater pooling of blood in the congested extremities. This might have resulted from a reduction of "post-arteriolar tone" in the congested limbs produced by these drugs. The increased vascular capacity would thus produce critical loss of blood volume from the central circulation. However, this possibility was disproven by actual measurement of blood volume trapped in the limbs which demonstrated that despite the appearance of hypotension no more blood was trapped out during the post-drug as compared to the control congestion periods.

Plethysmographic measurement of the increase in volume of the calf during congestion also failed to reveal a greater increase in calf volume after as compared with before administration of these agents. In several instances the *rate* of increase in calf volume was greater following the drug suggesting a decrease in arteriolar and/or post-arteriolar tone (or a failure of reflex arteriolar constriction in the limb). However, marked hypotension occurred before the *total* volume increase was as great as that achieved at the end of five minutes of congestion in the control period. Thus, the observed changes in calf volume provided additional confirmatory data that the collapse was not associated with increased amounts of blood trapped in the limbs.

The increased rate of blood loss to the extremities following these hypotensive agents may have contributed somewhat to the collapse reaction by producing a rapid decrease in venous return and hence of cardiac output. However, the venesection experiments demonstrated that increased rate of blood loss was not the major cause of the hypotensive reaction. Figure 2 shows that the arterial pressure fell despite gradual removal of 500 cc. of blood over a period of eight minutes, whereas a similar rate of removal in the control subjects of these venesection experiments had no significant effect on the arterial pressure.

Another possible explanation for the increased susceptibility to congestion collapse was that the hypotension was due to failure of homeostatic vasoconstriction either arteriolar, post-arteriolar or both, in areas other than in the congested limbs (12). The vascular system in essence is composed of a pump, an arterial conduit leading to a capillary reservoir and a return or venous conduit. The capillary reservoir which has a huge capacity in comparison to the total blood volume is capable of considerable variation in volume. If the reservoir were to enlarge to its full capacity blood would be lost from the large conduits, whereas if it became smaller an excessive amount of blood would be forced into the central circulation. If this closed system were static, blood loss would produce a fall in pressure throughout and a diminution of venous return. However, since the normal vascular system is dynamic, the capillary reservoir is capable within certain limits of reducing its capacity in proportion to the degree of blood loss. Thus, the venous conduit remains filled and venous pressure and return are maintained. Certain hypotensive agents by doing away with the ability to reduce the volume of the capillary beds convert the vasculature into a more static system with the result that during blood loss the pressure falls throughout and venous return fails.

If the collapse were due to failure of compensatory reactions to minor degrees of blood loss from the central circulation it would be expected that moderate blood loss by any route including venesection would produce a similar hypotensive reaction. After the administration of sodium nitrite, the mixed dihydrogenated alkaloids of ergot or hexamethonium, hypotension and collapse did occur following blood loss by venesection in amounts insufficient to induce significant hypotension in untreated individuals. These venesection experiments, therefore, provided direct evidence that the collapse during the post-drug congestion period was due to failure of compensatory vasoconstriction in the presence of relatively small amounts of blood loss.

The present data are not inconsistent with previous observations that sodium nitrite reduces "venous" (post-arteriolar) tone but does not prevent sympathetic vasoconstrictor reflexes involving the arterioles (10, 11). In the prior studies graded congesting pressures of 10 to 50 mm. Hg were applied for brief periods of only a few seconds to demonstrate the reduction in post-arteriolar tone (10). However, it must be emphasized that the congesting pressures of 60 to 100 mm. Hg used in the present experiments are far higher than those normally present in the capillaries and venules (13). When such pressures are applied for as long as five minutes maximum filling of the post-arteriolar vascular bed must occur whether or not sodium nitrite has been given. This accounts for the fact that no more blood was trapped out after as compared to prior to sodium nitrite. Collapse occurs in the post-nitrite congestion period not because of a greater total amount of blood pooled in the limbs but rather from failure of a compensatory reduction in the volume of the post-arteriolar vascular bed in areas other than in the congested limbs. Although this action of sodium nitrite is not definitely clarified the available data imply that while the drug does not prevent arteriolar constriction it must have some paralytic action on the post- arteriolar blood vessels (10). In this respect it differs from the sympatholytic agents which markedly inhibit reflex arteriolar constriction (11).

Perhaps of greater significance was the observation that withdrawal of a relatively small amount of blood by venesection, that is, 100 cc. or 2 to 3 per cent of the total blood volume, resulted in a perceptible decrement in arterial pressure after premedication with a hypotensive drug. This was particularly apparent after hexamethonium, a drug which apparently produces relatively complete sympathetic blockade in man (14). Such observations provide an index of the sensitivity of the homeostatic regulators of the circulation. These data suggest that in the normal, untreated individual losses of less than 5 per cent of the total blood volume stimulate compensatory decreases in the capacity of the vascular tree.

Following administration of adequate doses of the sympatholytic agents the subsequent level of arterial pressure depended in large measure on the position of the patient. A head down posture usually prevented marked hypotension while a slight head up tilt of the body precipitated collapse. In the supine patient following the administration of $C_6$ marked reductions in arterial pressure could be induced either by tilting, venous tourniquets or moderate degree of blood loss by venesection. These observations are consistent with the concept that the sympathetic vasoconstrictor nerves and associated medullary vasomotor centers are primarily concerned with the rapid reflex adjustment of the capacity of the vascular system, toward regulation of arterial pressure under stress, rather than in the primary regulation of the "basal" level of blood pressure.

The resistance of partially sympathectomized subjects to collapse during venous congestion appears most likely to be related to the mechanisms which result in a gradual restoration of vascular "tone" in denervated areas. This resumption of tone is seen in the disappearance of increased blood flow in the forearm (15), in the hand (16), and in the hepatic-portal circuit (17) after regional sympathectomy as well as the gradual diminution of postural hypotension in patients who have undergone extensive splanchnicectomy (18). The mechanism whereby vascular tone is regained is unknown (19), but apparently, as evidenced by the present experiments, it results in attainment of at least partial homeostasis without dependence on local sympathetic vasoconstrictor reflexes.

## SUMMARY AND CONCLUSIONS

1. The prior administration of sodium nitrite, the dihydrogenated alkaloids of ergot, hexamethonium, and to a lesser extent tetraethylammonium to normotensive or hypertensive patients resulted in a state of increased susceptibility to the development of hypotension and collapse when the limbs were congested with venous tourniquets.
2. Veratrum viride and sodium amytal did not produce this effect.
3. Measurement of the volume of blood trapped in the limbs (dye method) and the increase in volume of the calf (plethysmograph) indicated that the hypotension and collapse were not due to pooling of excessive amounts of blood in the congested limbs.
4. The hypotension and collapse were due to failure of compensatory vasoconstriction in other areas than in the congested extremities following moderate blood loss. This was demonstrated by the observation that after the administration of sodium nitrite, the mixed dihydrogenated alkaloids of ergot or hexamethonium hypotension frequently with collapse occurred during venesections of only 250 to 525 cc. of blood.
5. After these drugs loss of as little as 2 to 4 per cent of the total blood volume frequently resulted in perceptible decrements of arterial pressure. Such observations illustrate the extreme sensitivity of the homeostatic vasoconstrictor mechanisms in the normal (untreated) individual.
6. Patients who had previously undergone lumbodorsal or transthoracic sympathectomy were more resistant to hypotension during the post-drug congestion period than were non-sympathectomized subjects. This resistance probably was related to the well known return of independent vascular tone in sympathectomized areas.

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# EFFECTS OF ADRENOCORTICOTROPIC HORMONE AND CORTISONE IN PATIENTS WITH TUBERCULOSIS[1, 2]

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(Submitted for publication January 5, 1951; accepted, March 2, 1951)

## INTRODUCTION

In the early reports on the administration of adrenocorticotropic hormone and cortisone to patients with various disease states, there were described a number of effects, the reproduction of which might conceivably alter the course of infections in man. Among these effects were defervescence, decrease in edema and inflammation, change in antibody production, and alteration in the state of hypersensitivity to bacterial products. Clinical evidence of such effects was provided by Finland, Kass and Ingbar (1) who showed that adrenocorticotropic hormone may effect striking alterations in the course of pneumococcal and primary atypical pneumonia. Moreover, equally striking changes were observed by Freeman and his coworkers in two patients with pulmonary tuberculosis (2). The modification of response to infection, by an agent which presumably acts solely upon the host, thus presents an opportunity for further evaluation of the participation of host mechanisms in tuberculous disease.

There are relatively few forms of tuberculosis in humans appropriate for such a study. A serious form of progressive disease, with a reasonably predictable course, is necessary. It is desirable to avoid reliance upon chest roentgenograms as the sole means of evaluating changes in a tuberculous lesion. In this situation, the extent of viable tissue within a lesion must be determined by a procedure which is highly subjective before proper evaluation of subsequent changes within the lesion can be accomplished. Although the accuracy of the method is increased by having the roentgenograms interpreted independently by several competent observers, the interpretations frequently do not reflect the course of the disease as judged by other criteria (3). Such is not the case, however, when a lesion is available in which changes occurring after the introduction of a variable can be easily observed by more direct visualization. For these reasons, a special effort was made to include patients with active laryngeal tuberculosis in this study.

## COMPOSITION OF CLINICAL SERIES AND METHODS OF STUDY

Seven patients with far advanced pulmonary tuberculosis were selected for study. Four of the seven had active laryngeal tuberculosis with lesions of the larynx which could be readily visualized by indirect laryngoscopy. A fifth patient had tuberculous empyema on the right side and was considered to be in a terminal phase of her illness. The strains of *M. tuberculosis* isolated in cultures of specimens of sputum from four of the seven patients were highly resistant to dihydrostreptomycin *in vitro*.

The course of tuberculous illness in each patient was evaluated prior to hormone administration from the available clinical, roentgenographic, bacteriologic and immunologic data. Whenever possible, a preliminary observation period of seven to ten days was undertaken. Each patient then received 100 mgm. of hormone intramuscularly in four equally divided doses at six hour intervals for an initial ten day period. Four of the patients received adrenocorticotropic hormone[4] and three received cortisone.[4]

Throughout the study all patients were maintained at rest in bed. They received diets of their own selection and an estimate was made of actual caloric and protein intake. The pulse rate and rectal temperature were recorded at two hour intervals. In addition to customary bedside

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[1] Presented in part before The American Society for Clinical Investigation, Atlantic City, N. J., May 1, 1950.

[2] This study was supported in part by grants from The Division of Research Grants and Fellowships, National Institutes of Health, United States Public Health Service; the Lederle Laboratories Division, American Cyanamid Company, Pearl River, N. Y.; Chas. Pfizer & Company, Brooklyn.

[3] Post-doctorate Research Fellow, National Institutes of Health, U. S. Public Health Service.

[4] The adrenocorticotropic hormone used in this investigation was obtained through the courtesy of Dr. John Mote, Armour and Co., Chicago, Ill. The cortisone was obtained through the courtesy of Dr. James M. Carlisle, Merck and Co., Rahway, N. J.

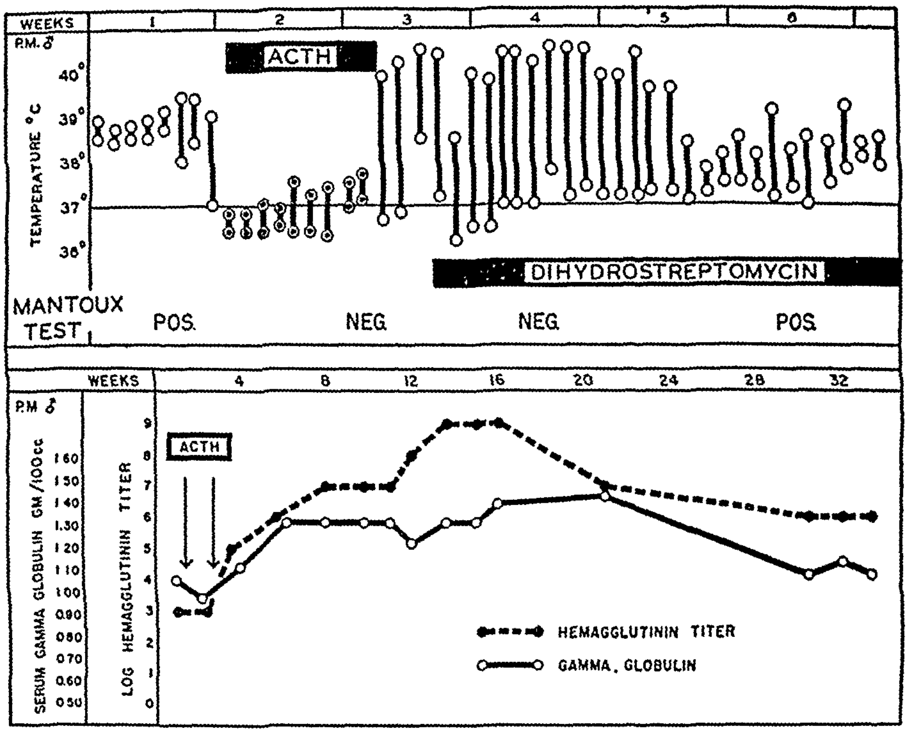

FIG. 1. PATIENT P. M., AGE 49 YRS.

In the upper portion of the chart are shown the daily range of temperature and the results of tests for cutaneous hypersensitivity to tuberculoprotein. Note the rapid defervescence following the start of ACTH administration, and the abrupt return of fever with wide fluctuations after stopping ACTH. The return of cutaneous hypersensitivity to tuberculoprotein coincides with the fall in fever while under treatment with dihydrostreptomycin. In the lower portion of the chart are shown the alterations in the values obtained for concentrations of serum gamma globulin and in the titer of hemagglutinating antibodies.

observations, indirect laryngoscopy was performed on each patient with laryngitis. Drawings were made of the laryngeal lesions as seen in the laryngeal mirror, at intervals determined by change in the appearance of the lesions. Serial chest roentgenograms, using a standardized technique, were obtained at 72 hour intervals.

Twenty-four hour aliquots of continuous sputum collections were measured for volume and the character of the specimen noted. The aliquots were then digested, centrifuged and direct smears were made of the concentrate. Duplicate smears were stained with carbol fuchsin by the Ziehl-Nielsen technique and with Sudan black B (4). Petragnani and oleic acid-albumin media were used for cultivation of the *M. tuberculosis* from the concentrated specimens of sputum. The colonial morphology of the bacilli growing on oleic acid-albumin medium was examined for the characteristics known to be associated with various degrees of virulence (5). Organisms obtained from each patient at weekly intervals were grown in Tween[5]-albumin medium and were tested for sensitivity to dihydrostreptomycin.

Observations on the cutaneous hypersensitivity to tuberculin were made using an amount of tuberculoprotein, standardized individually for each patient, sufficient to obtain an unequivocally positive reaction. An initial reaction of more than 10 mm. in diameter showing erythema and definite edema was obtained in five patients using 0.00002 mgm. of Tuberculin, Purified Protein Derivative[6] (First Test Strength). In one patient (P. M.), 0.005 mgm. of Tuberculin, Purified Protein Derivative (Second Test Strength) was required to produce a reaction of this magnitude. The reactions were examined and the outlines traced on cellophane at 48 and 96 hours after intracutaneous injection of the forearm.

Specimens of serum were obtained at frequent intervals from each patient for estimation of tuberculin hemagglutinating antibody titer as described by Middlebrook and Dubos (6) and the concentration of serum gamma globulin by the method of Kunkel (7). Bone marrow examinations with particular reference to the plasma cell count were done as described by Good and Campbell (8). Other laboratory examinations were made in each patient and gave results comparable to those reported in other studies with these compounds.

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[5] "Tween 80" is the trade name of the polyoxyethylene derivative of sorbitan monooleate, obtained from the Atlas Powder Company, Wilmington, Del.

[6] The Tuberculin, Purified Protein Derivative used in this investigation was obtained from Parke, Davis and Co., Detroit, Mich.

## RESULTS

*Course of illness:* In five patients the onset of hormone administration marked the inception of a series of profound changes, the first of which were evident as early as six to eight hours after the initial dose. In all patients, a strikingly rapid disappearance of the signs and symptoms of acute illness occurred and persisted throughout the period of hormone administration. Following the cessation of hormone administration, however, a quick return to the previous state of illness was observed.

In three of the four febrile patients defervescence was complete within six hours of the start of hormone administration (Figures 1–3). This process required 72 hours for completion in one patient (Figure 4). All patients remained essentially afebrile until the cessation of hormone administration. Defervescence was accompanied by a feeling of increased strength and desire for physical activity. Appetite increased markedly with concurrent rise in the caloric and protein intake.

In the four patients with laryngitis improvement in phonation and disappearance of dysphagia were evident by the end of the initial 48 hour period of hormone administration. Thereafter, a progressive increase in the volume of the voice and improvement in its quality ensued. Discomfort from dyspnea and cough was markedly relieved although the volume of the sputum was not significantly decreased. All of the patients experienced some degree of a sense of well-being which in some amounted to euphoria.

The cessation of hormone administration was followed by abrupt return of the signs and symptoms of acute illness. The manifestations of acute illness immediately following the end of hormone administration were at least equal in severity to those present before hormone administration and

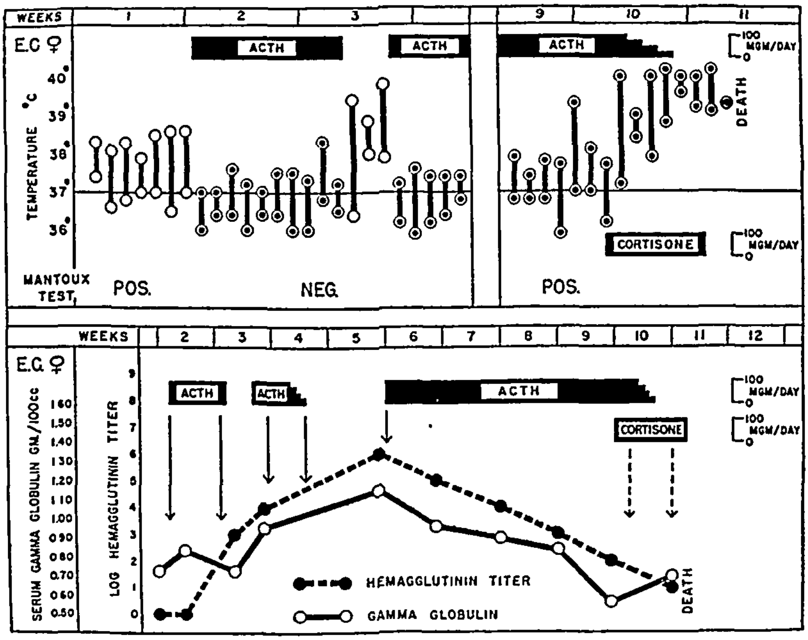


FIG. 2. PATIENT E. C., AGE 21 YRS.

Note the rapid defervescence and abrupt return of fever at the onset and end, respectively, of the initial course of ACTH administration. Following reinstitution of ACTH administration prompt defervescence occurred. Escape from the controlling effects of ACTH occurred later with return of fever and cutaneous hypersensitivity to tuberculoprotein despite the addition of cortisone. In the lower panel are shown the rises in serum gamma globulin values and in titer of tuberculin hemagglutinating antibodies following the initial course of administration of ACTH. Note the progressive decline of both serum gamma globulin values and hemagglutinin titer during prolonged ACTH administration.

in four patients (P. M., E. C., S. H., and T. B.), were more severe. Fever occurred within 18 hours after the last dose of adrenocorticotropic hormone. Following the cessation of cortisone administration, however, fever occurred on the second and sixth day (Figures 4 and 5) or was not a prominent feature in the post-hormone course (Figure 6).

The fever following the end of hormone administration was self-limited in two patients who were essentially afebrile prior to hormone administration (Figures 5 and 7). In one patient (Figure 1) despite the institution of dihydrostreptomycin therapy soon after stopping adrenocorticotropic hormone, fever continued for four weeks. It may be noted here that in this patient, subsidence of fever coincided with the return of cutaneous hypersensitivity to tuberculoprotein. Significant defervescence was not observed during dihydrostreptomycin administration in another patient (Figure 4). Subsequently, the strain of *M. tuberculosis* isolated from cultures of the sputum of this patient was found to be resistant *in vitro* to dihydrostreptomycin.

*Laryngeal lesions:* Observations of the changes in the laryngeal lesion was made by daily indirect laryngoscopic examination in the four patients with tuberculous laryngitis. In all patients a similar sequence of changes was observed. The changes noted in the laryngeal lesion of one patient (P. M.) are depicted in Figure 8 and may be considered as characteristic of the changes observed in all patients studied.

In this patient, initial examination of the left side of the larynx demonstrated ulceration with partial replacement by granulation tissue of the lateral portion of the arytenoid tubercle, aryepiglottic fold and true vocal cord, in addition to absence of one-half of the epiglottis. The entire right half of the larynx was edematous with marked inflammation and both true vocal cords were irregular.

Forty-eight hours after the beginning of hormone administration, edema and inflammation had subsided almost completely. In the ulcerated le-

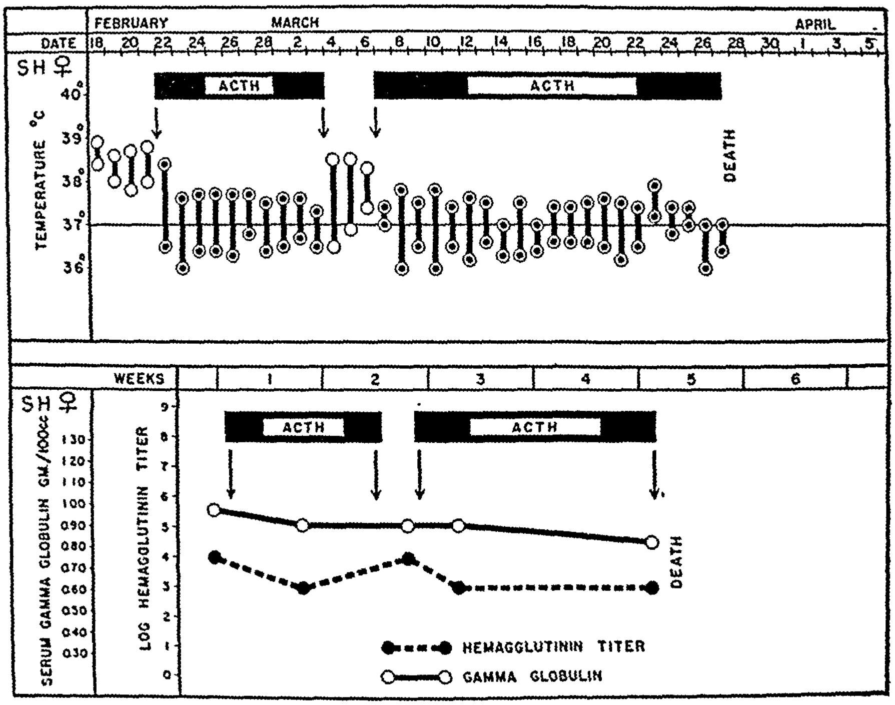


FIG. 3. PATIENT S. H., AGE 36 YRS.

Rapid defervescence following the onset of the initial course of ACTH administration and abrupt return of fever at the end of this period of administration are shown. Upon reinstitution of ACTH administration, defervescence again occurred rapidly. Death ensued during prolonged ACTH administration without return of fever. In this patient no significant alterations occurred in serum gamma globulin values or in titer of hemagglutinating antibodies following the initial ten day course of ACTH or upon prolonged ACTH administration.

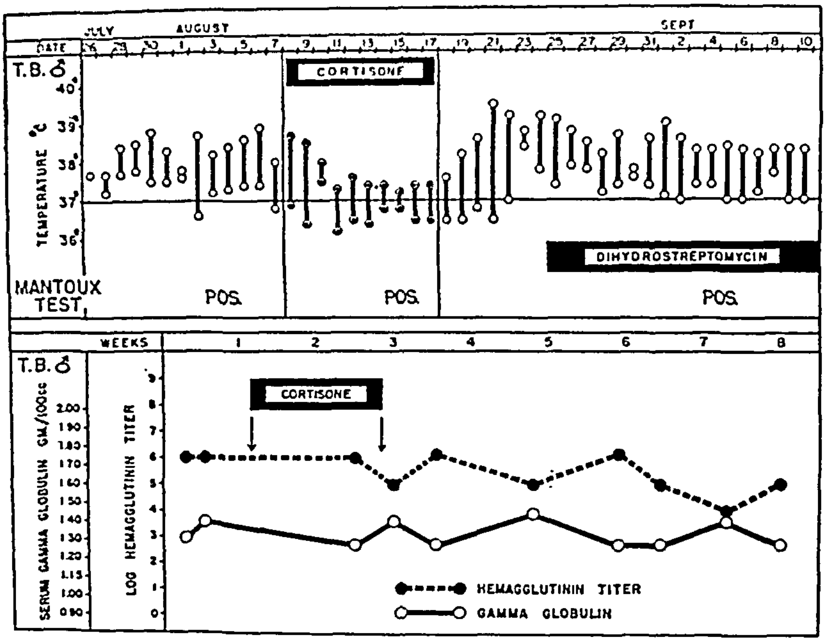


FIG. 4. PATIENT T. B., AGE 40 YRS.

Following the onset of cortisone administration, defervescence required 72 hours for completion. Fever slowly recurred following cessation of cortisone administration and persisted despite dihydrostreptomycin therapy. Subsequently the strain of *M. tuberculosis* isolated from culture of the sputum was found to be resistant to dihydrostreptomycin. Reversal of cutaneous hypersensitivity to tuberculoprotein was not observed in this patient. No significant alterations occurred in serum gamma globulin values or in titer of the tuberculin hemagglutinating antibodies.

sions, a border of epithelium could be visualized progressing from the edges of the ulcerated area as the granulation tissue subsided. By the ninth day of hormone administration, the epidermization had covered approximately 85 per cent of the previously ulcerated area.

Within 48 hours after completion of hormone administration, edema and inflammation had returned although no regression of epithelium was observed. Subsequently, no significant alterations in the appearance of the laryngeal lesion attributable to hormone effect were observed.

Complete healing with scarring occurred following the institution of dihydrostreptomycin therapy in two patients whose sputum contained strains of *M. tuberculosis* found to be sensitive to the drug *in vitro*.

*Lung lesions:* Serial roentgenograms, interpreted independently by several observers, revealed definite changes in the appearance of the lung lesions. These changes formed a consistent pattern in five of the seven patients. A representative example of the changes is shown in Figure 9 portraying enlarged segments of chest roentgenograms from one of the patients (P. M.).

In general, during the administration of adrenocorticotropic hormone the appearance of lung lesions became more translucent with better delineation of the individual lesions. Within 48 hours after cessation of hormone administration definite increase in the density of the lesions was apparent. No patient in this series has been observed without additional therapeutic measures for a sufficient time beyond this point to evaluate the roentgenographic changes which might result solely from hormone administration.

Dihydrostreptomycin therapy was instituted in this patient (P. M.) and continued for 111 days. The increase in density reached a maximum in the fourth week, the lesions thereafter became more translucent. It is of interest that in this patient the onset of defervescence and the return of cutaneous hypersensitivity to tuberculoprotein also occurred during the fourth week after the end of

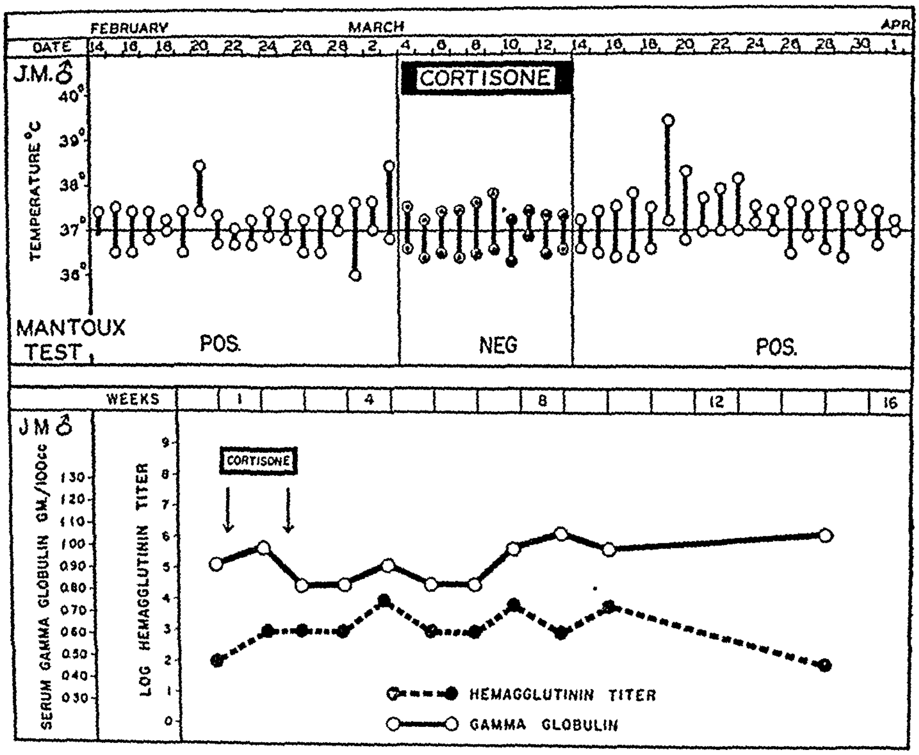


FIG. 5. PATIENT J. M., AGE 51 YRS.

Fever occurred on the sixth day after cessation of cortisone administration and gradually subsided over a 48 hour period. Reversal of cutaneous hypersensitivity to tuberculoprotein is illustrated in the upper panel. No significant alterations in serum gamma globulin values or in titer of tuberculin hemagglutinating antibodies occurred in this patient.

hormone administration. Progressive decrease in the size and density of the roentgenographic appearance of the lung lesions ensued, marked clearing being noted seven months after hormone administration (Figure 9).

Resorption of a small purulent intrapleural exudate was observed in one patient during the administration of adrenocorticotropic hormone. As this patient (S. H.) was in the terminal phase of her illness, prolonged observation after hormone administration was impossible. No well-defined changes in the roentgenographic appearance of the lung lesions occurred in two patients, one receiving adrenocorticotropic hormone (A. R.) and the other cortisone (J. M.).

Roentgenographic evidence of dissemination of the tuberculous process was not obtained in any patient.

*Skin hypersensitivity to tuberculoprotein:* Repeated observations on the skin hypersensitivity to tuberculoprotein were made. In each of the six patients tested an amount of tuberculin (Purified Protein Derivative) was used sufficient to elicit an unequivocally positive reaction prior to the administration of hormone. Reversal of cutaneous hypersensitivity to this individually standardized amount of tuberculoprotein was observed during hormone administration in three of the six patients tested. This reversal of a previously positive response was maintained for three to four weeks after the cessation of hormone administration in the two patients followed from this standpoint. In three patients (Figures 4, 6 and 7) negative reactions were not obtained.

It is of considerable interest that despite the continued absence of cutaneous hypersensitivity to the test dose of tuberculoprotein the acute manifestations of tuberculous illness reappeared following cessation of hormone administration in the three patients in whom reversal of the tuberculin skin test was obtained.

*Serum gamma globulin and tuberculin hemagglutinating antibodies:* The substantially elevated serum gamma globulin values initially present in one patient (Figure 7) decreased toward the normal range during and immediately following adrenocorticotropic hormone administration. Subsequently, the values rose, reaching the high level present initially by the end of four weeks, and rose even further thereafter. Aside from this instance, significant variations in the serum gamma globulin values did not occur during the ten day period of hormone administration. Likewise, the titers of the tuberculin hemagglutinating antibody were not significantly changed during this period.

Within three weeks after completion of hormone administration, however, three patients had significant increases both in the concentration of serum gamma globulin and in the titer of the tuberculin hemagglutinating antibodies (Figures 1, 2 and 6). The rises were sustained in one patient (P. M.) in whom the values reached a maximum between the 14th and 16th weeks and declined thereafter. Although significant increases in both serum components were noted in another patient (R. R.) immediately following cessation of cortisone administration, the evolution of the changes has been observed for only a short period of time. In the third patient (E. C.) reinstitution of adrenocorticotropic hormone at the peak of the rise in serum gamma globulin and hemagglutinating antibody titer was followed by progressive decline toward the original values.

*Prolonged hormone administration:* Two of the most critically ill patients (E. C. and S. H.) received additional adrenocorticotropic hormone to relieve the extreme prostration which accompanied the return of fever. The strains of *M. tuberculosis* recovered in cultures of the sputum of both were highly resistant to dihydrostreptomycin *in vitro.* Upon reinstitution of 100 mgm. per day of adrenocorticotropic hormone, the onset of defervescence was again rapid and complete and the signs and symptoms of acute illness disappeared promptly.

In one of these patients (E. C.) an unsuccessful attempt was made to decrease the severity of the post-hormone manifestations of illness by gradual reduction of the amount of hormone administered over a four day period. Subsequently, reinstitution of adrenocorticotropic hormone was necessary to alleviate the manifestations of acute illness. During the fourth consecutive week of hormone

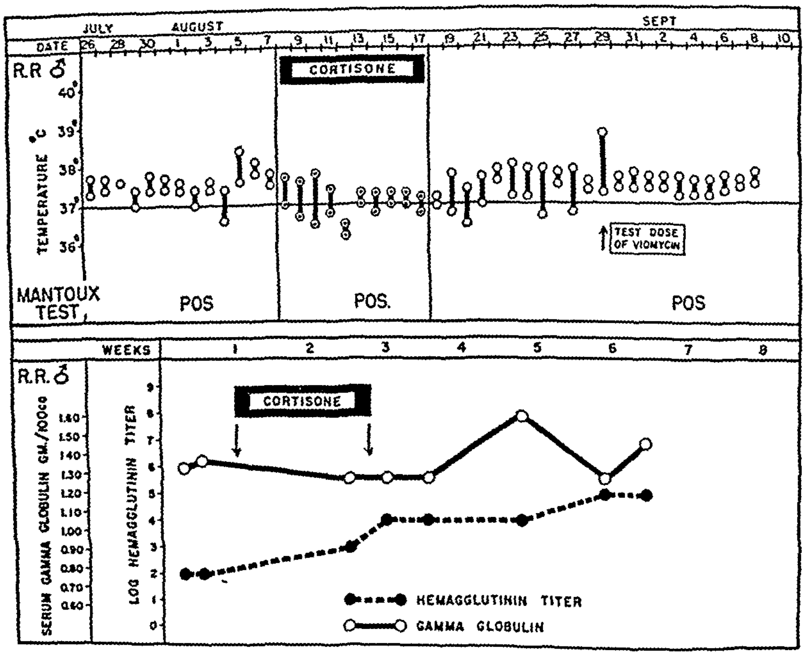


Fig. 6. Patient R. R., Age 25 Yrs.

Slight decrease in the daily temperature range during cortisone administration is shown in the upper panel. No post-hormone fever attributable to the cessation of cortisone administration occurred in this patient. Reversal of cutaneous hypersensitivity to tuberculoprotein was not observed. Significant increases in both serum gamma globulin values and in titer of hemagglutinating antibodies immediately following cessation of cortisone administration are shown.

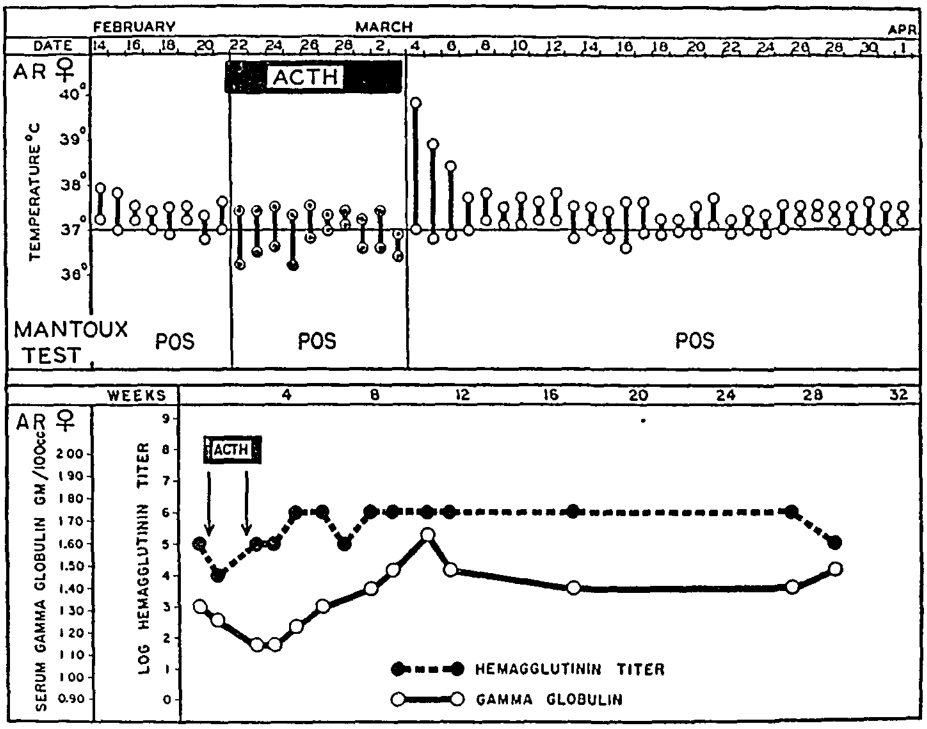


Fig. 7. Patient A. R., Age 39 Yrs.

Note the occurrence of fever immediately following cessation of ACTH administration in this patient who was essentially afebrile prior to hormone administration. Reversal of cutaneous hypersensitivity to tuberculoprotein was not observed. Decrease in serum gamma globulin values occurred during and immediately after cessation of ACTH administration, and subsequently rose to values higher than the initial level.

administration, in the ninth week of study, hallucinations and delusions appeared abruptly. The following week fever recurred with gradual return of malaise, cough, and increasing prostration; cutaneous hypersensitivity to the test dose of tuberculoprotein also reappeared (Figure 2). The patient's condition rapidly became worse, despite continuation of adrenocorticotropic hormone and the addition of 100 mgm. per day of cortisone. Gradual reduction and cessation of adrenocorticotropic hormone administration failed to produce evidence of improvement. Cortisone administration was maintained for four days without apparent change in the patient's clinical condition and was discontinued in the fifth consecutive week of hormone administration. Thirty-six hours following cessation of cortisone administration, this patient expired.

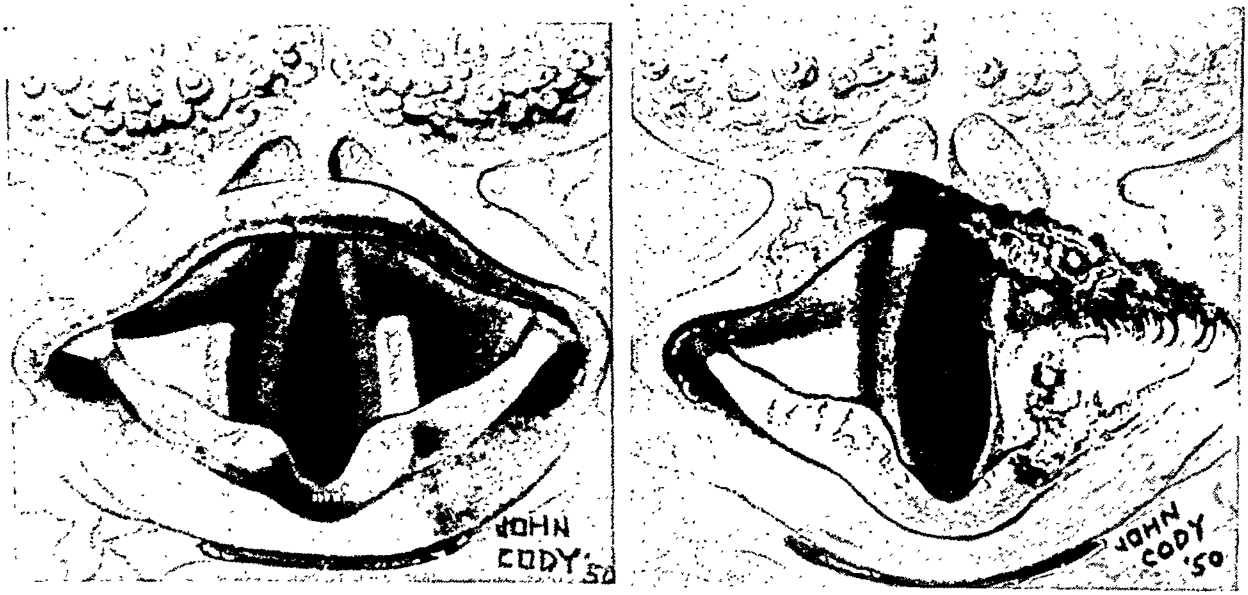
Reinstitution of adrenocorticotropic hormone in another patient (Figure 3) was followed by a succession of events similar to those described above, although less pronounced. Marked symptomatic improvement and defervescence were gradually supplanted by euphoria and general weakness. Death occurred on the 21st consecutive day of hormone administration in the fifth week of study without recurrence of prostration or fever.

Post-mortem examination revealed no evidence of recent dissemination of the tuberculous process or significant alteration in the histopathology of the tuberculous process in either patient. The adrenal cortices of both patients were uniformly thickened and the glands were increased in weight. It is of interest that a small nodule of caseation necrosis was found at the surface of the right adrenal gland in one patient (S. H.).

*Subsequent course:* Two of the patients (P. M. and J. M.) have shown steady, favorable progress following the institution of dihydrostreptomycin and para-amino-salicylic acid administration. Sputum concentrates and cultures no longer show the presence of *M. tubercuolsis.* Marked clearing in the chest roentgenogram of P. M. has occurred as previously noted. The activity of his tuberculous process being considered arrested, J. M. has been discharged from the hospital.

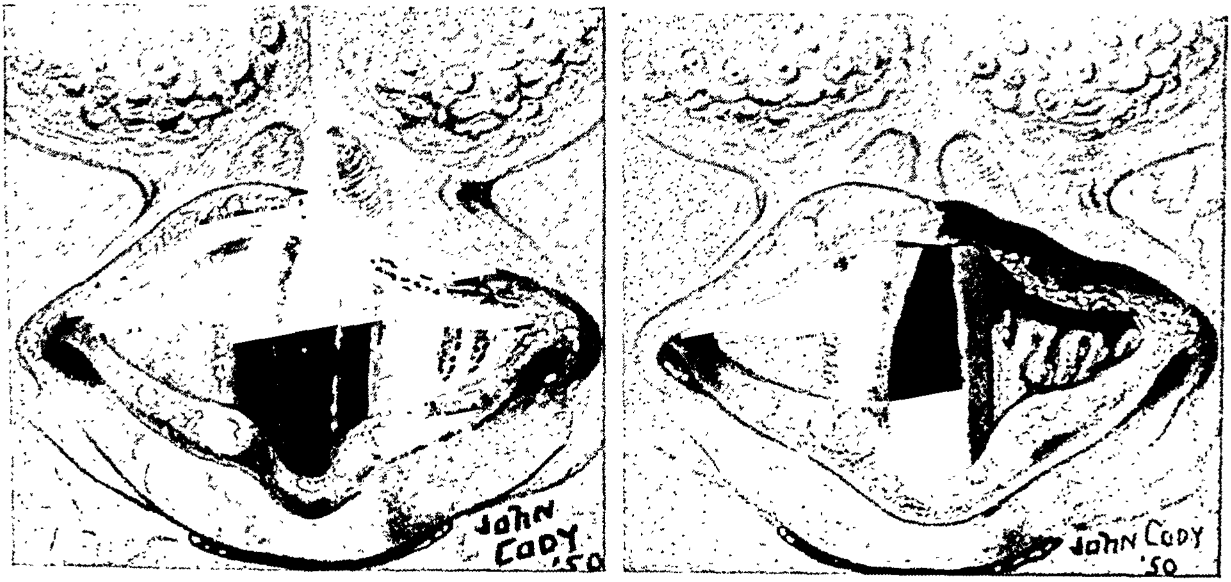
One of the patients (A. R.) whose sputum contained a strain of *M. tuberculosis* found to be resistant to dihydrostreptomycin *in vitro*, has been observed for ten months while receiving pneumoperitoneum therapy and bed rest. Her clinical status, though not conspicuously changed, has shown slight improvement. No significant alteration of her laryngeal lesion has been observed since the changes noted immediately after cessation of adrenocorticotropic hormone administration.

The two remaining patients (T. B. and R. R.) have not been observed for a sufficient period of time following the administration of cortisone to permit final evaluation of the influence of the hormone on the course of the infection. No dele-



*a*) Normal larynx as seen in a laryngeal mirror.

*b*) P. M.—Larynx before ACTH.



*c*) P. M.—Larynx after nine days of ACTH.

*d*) P. M.—Larynx two days after cessation of ACTH.

FIG. 8. PATIENT P. M. CHANGES IN THE LARYNGEAL LESIONS

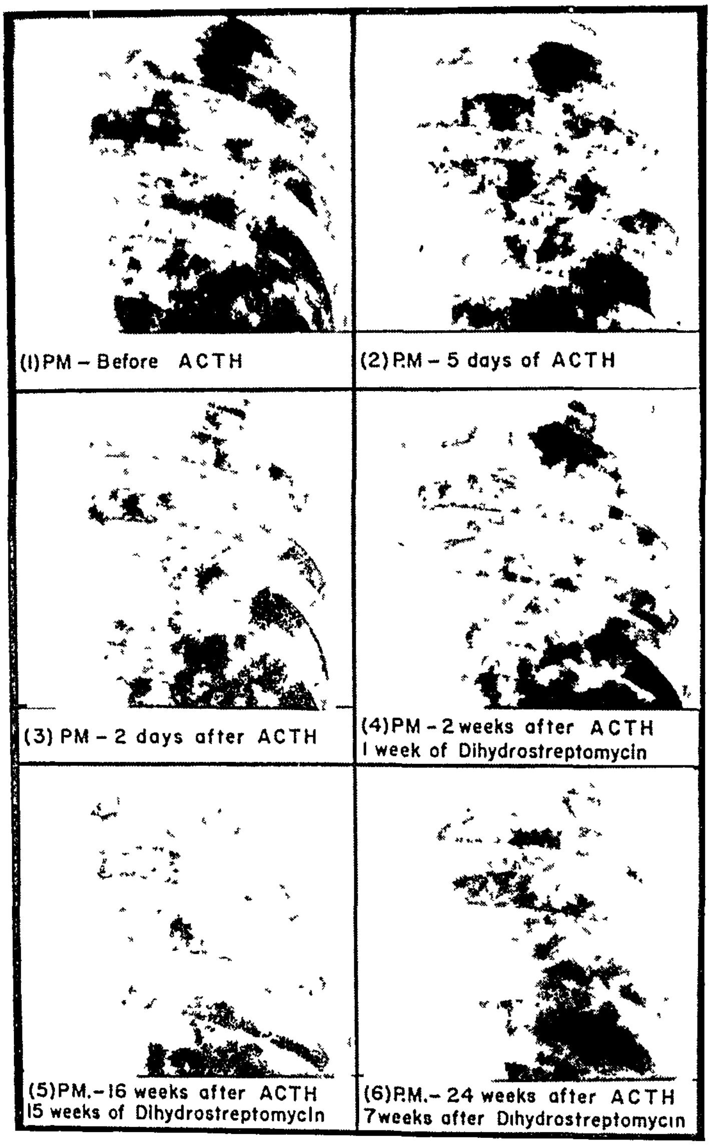

(1) PM – Before ACTH
(2) P.M – 5 days of ACTH
(3) PM – 2 days after ACTH
(4) PM – 2 weeks after ACTH
I week of Dihydrostreptomycin
(5) PM. – 16 weeks after ACTH
15 weeks of Dihydrostreptomycin
(6) P.M. – 24 weeks after ACTH
7 weeks after Dihydrostreptomycin

FIG 9 PATIENT P M. CHANGES IN THE ROENTGENOGRAPHIC APPEARANCE OF THE LUNG LESIONS

(*1*) An enlarged segment of the initial chest roentgenogram. (*2*) Note slight decrease in density of the lung lesions (*3*), (*4*) Note marked increase in density in the appearance of the lung lesions. (*5*), (*6*) Marked clearing of lung lesions after dihydrostreptomycin.

terious effect of cortisone administration, however, has been noted during the four month period of observation.

*Tubercle bacilli:* Tubercle bacilli were discharged in the sputum of all patients without significant fluctuations in number throughout the period of observation. Retention by the tubercle bacilli of initial staining properties with carbol fuchsin and Sudan black B and of initial *in vitro* sensitivity to dihydrostreptomycin was observed. Colonies grown on oleic acid-albumin attained the morphologic characteristics attributed to virulent organisms by Middlebrook, Dubos, and Pierce (5) in all specimens examined. Therefore, no bacteriologic evidence of alteration in the tubercle bacilli isolated from sputum or empyema was obtained.

### COMMENT

From the observations reported above, it appears that abrupt and profound changes in the course of tuberculous illness may be brought about by the introduction of hormones which presumably affect only the host.

During the period of administration of these hormones, rapid amelioration and subsequent disappearance of the constitutional manifestations of acute illness were witnessed. Complete defervescence was accompanied by a seemingly improved state wherein increase in strength, appetite, and desire for activity occurred. It is of particular interest to note that the onset of the seemingly improved state was equally evident in two patients (J. M. and A. R.) who were essentially afebrile prior to hormone administration. Improvement in phonation and disappearance of dysphagia was striking as was the recession and subsequent quiescent appearance of the laryngeal lesions. Decrease in density of the lung lesions seen on chest roentgenogram was noted in five patients. Reversal of cutaneous hypersensitivity to an arbitrarily selected amount of tuberculoprotein was observed in three of the six patients tested.

The termination of the state of apparent improvement following the withdrawal of hormone administration was equally evident. The signs and symptoms of acute illness which had been alleviated, reappeared abruptly, and were equal to or even more severe than those present before hormone administration. The return of edema and inflammation in the laryngeal lesions also occurred promptly as did the increase in density of lung lesions seen on chest roentgenograms. The swift return of the manifestations of acute illness contrasted sharply with the relatively slow return of cutaneous hypersensitivity to tuberculoprotein and rise in the titer of the tuberculin hemagglutinating antibodies observed several weeks after cessation of hormone administration.

The abrupt disappearance of the manifestations of illness induced by adrenocorticotropic hormone and cortisone is of considerable interest in regard to the nature of the toxicity of tuberculosis. The mechanisms by which this infection produces fever and other manifestations of illness are not completely understood. It has been generally believed that the tissue damage and the clinical illness are, in large part, consequences of the interaction of previously sensitized tissue and the absorbed products from tubercle bacilli (9*a*). *In vitro* studies have been demonstrated that cells of the hypersensitive body are damaged and killed by amounts of tuberculoprotein that are harmless to cells in the absence of hypersensitivity (9*b*). Whether the living tubercle bacillus exerts a primary toxic effect upon host tissue is not definitely established (10). Diminution in the manifestations of illness might be expected if, by alteration in the reactivity of host tissue, some degree of desensitization to the products of infection was obtained. Conversely, upon reappearance of the effects of hypersensitization their return would not be surprising.

From the observations reported here, it seems clear that reactivity of host tissues sufficient to cause severe symptoms of tuberculous illness may be present without the presence of a positive Mantoux test. Moreover, distinct symptomatic improvement was observed after the start of hormone therapy in those patients whose Mantoux test remained positive. The inability to utilize clinical quantitation and subsequent correlation of local and systemic tissue hypersensitivity to tuberculoprotein makes speculation on this aspect inadvisable.

The sudden suppression of the clinical evidence of toxicity observed to occur at the start of hormone administration may be compared to the similar state observed in patients with tuberculosis under treatment with streptomycin. Loss of so-called "toxemic" symptoms may occur in both situations and although improvement following the initiation of streptomycin therapy may be observed as early as the third day of therapy, it has not been observed to be as rapid in onset nor does its speed approach that of the changes occurring in the patients reported above. Thus the same phenomenon, sudden suppression of the clinical evidences of toxicity, may be produced on the one hand, by actions exerted principally upon the parasite and on the other, by action exerted solely through the host.

The course of the two patients who expired suggests that the changes effected by a fixed amount of adrenocorticotropic hormone are temporary and cannot be sustained, even with continued administration. Whether modification of the amount of hormone administered, alteration in the frequency of administration, or the combination with other agents will alter this situation is yet to be determined.

## SUMMARY

The effects of adrenocorticotropic hormone and cortisone on the course of tuberculous illness has been studied in seven patients with far advanced pulmonary tuberculosis. It was found that abrupt and profound changes in the course of the illness were brought about by administration of these hormones. These changes were in the nature of defervescence, improvements in the sense of well-being, increased strength, improvement in appetite, as well as decrease in the edema and in the amount of exudate in the local lesions of the larynx. In all patients the improved state was temporary and, as might be anticipated, could not be indefinitely maintained by further administration of the hormones.

Observations were also made on the changes in cutaneous hypersensitivity to tuberculoprotein, in concentration of serum gamma globulin and in the titer of tuberculin hemagglutinating antibodies. Reversal of cutaneous hypersensitivity to an arbitrarily selected amount of tuberculoprotein during hormone administration was observed in three of the six patients tested. Within three weeks after completion of hormone administration significant increases both in concentration of serum gamma globulin and in the titer of tuberculin hemagglutinating antibodies were noted in three of the seven patients.

Interpretation of these temporary alterations in the disease state in terms of ultimate effect in such a chronic infection must await further study.

## ACKNOWLEDGMENTS

The authors are indebted to Dr. Sidney Rothbard and Dr. Ralph Engle for their cooperation in this investigation.

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# AGING AS A FACTOR IN THE RENAL HEMODYNAMIC CHANGES INDUCED BY A STANDARDIZED PYROGEN

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(Submitted for publication September 25, 1950; accepted, March 6, 1951)

The functional and anatomic changes of the kidney associated with increasing age have been demonstrated convincingly. The evaluation of renal function in aged persons has demonstrated a progressive decrease in the urea clearance (1), glomerular filtration rate, effective renal plasma flow, and renal tubular transfer maxima (2, 3). These decreases in function fit well with the anatomic studies which hold that the degree of renal arteriosclerosis present in the aging population is sufficient to account for the decrease in renal parenchyma on a basis of restricted blood supply (4–7).

The anatomic studies also agree that the predominant picture of arterial degeneration is that of intimal thickening (lumen attenuation and occlusion) in the larger arteries (interlobar and interlobular) with progressively less sclerosis in the vessels as the observations extend toward the glomerulus. Even in the age group beyond 80 years, Bell notes that only about 20 per cent of the renal arterioles are involved in the sclerotic process, and this involvement is not severe (7). In his study of the relationship of age to renal atrophy in non-hypertensive individuals free from renal disease, Bell states, "Areas of atrophy are wedge-shaped and are caused by intimal atherosclerosis with occlusion of fairly large branches of the renal artery."

The present study is concerned with an evaluation of the functional status of the renal arterioles in an aging population free of hypertension and demonstrable renal disease. Since it is believed that the increase in renal blood flow following the intravenous administration of a pyrogen is in large part mediated through a decrease in renal arteriolar tonus (8), we have employed a standardized pyrogen test to assay the capacity of the renal arterioles to dilate.

## METHODS

*Subjects.* The 54 male subjects studied were divided into three age groups: young (Y), age range 20–49, mean age 36.6 years, N = 20; middle (M), age range 50–69, mean age 58.8, N = 20; and old (O), age range 70–84, mean age 76.9 years, N = 14. The broad 30-year age span of the young group was so selected because the downward age trend in renal function is not significant until after the age of 50 (2). That this finding applied well to the present group is indicated by the similarity of the mean resting effective renal plasma flows for two subgroups: age 20–34, 609 cc./min./1.73 m.$^2$; age 35–49, 599 cc./min./1.73 m.$^2$

The subjects were patients on the acute and chronic wards and residents of the Infirmary (Home for the Aged) of the Baltimore City Hospitals. All patients studied were ambulatory.

Except as noted, subjects were selected who met the following criteria: *a*) absence of history of physical evidence of renal disease, hypertension, or congestive heart failure, *b*) urinalyses (two or more) negative for protein by the heat and acetic acid coagulation test and for significant microscopic abnormalities, *c*) blood hemoglobin greater than 12 grams per cent, *d*) absence of electrocardiographic evidence of arrhythmia or A–V conduction defect, *e*) blood urea nitrogen within the limits for the different age groups as reported by Lewis and Alving (1), and *f*) basal diastolic blood pressure below 90 mm. Hg. No limits were placed on the systolic blood pressure, which ranged from 100 to 181 mm. Hg. In the old group, three of the subjects, though normotensive at the time of testing, showed cardiac enlargement and previous hypertensive blood pressure readings. Also in the old group an additional two subjects showed signs of congestive failure. However, the mean baseline renal function values and the mean renal hemodynamic response for these individuals were the same as the mean for the other nine subjects in this group.

Additional clinical examinations were made which were not used for exclusion of subjects. Heart size was estimated by x-ray; seven subjects were included who showed cardiac enlargement (cardiac transverse diameter 10–20 per cent in six cases and 32 per cent in one case above normal for their height and weight). Electrocardiographic abnormalities suggestive of myocardial disease were noted in 11 subjects, eight of whom were in the old group. Palpable thickening and diminished pulsation of the brachial, radial, dorsal pedal, and posterior tibial arteries were noted. Retinal arteriosclerosis was graded by an ophthalmologist.

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*Test procedure.* Subjects were studied in the post-absorptive state beginning at 8 a.m. Aminopyrine (0.6 gram) was given by mouth at four-hour intervals from 4 p.m. on the day preceding the test until 8 p.m. on the day of the test. Using the renal clearance technique of Goldring and Chasis (9) eleven 20-minute urine collection periods were carried out starting immediately after the intravenous administration of 50,000,000 killed typhoid organisms (0.05 cc. TAB vaccine). Blood samples were obtained through an indwelling femoral artery needle.

The sphygmomanometric blood pressure and the pulse rate by one-minute radial artery counts were determined at the fifth and 15th minute of each period. The average of the two determinations was recorded as the value for the period. Oral temperature was determined hourly during and for six to eight hours after the test.

*Chemical methods.* Inulin was determined in blood and urine by the method of Harrison (10). PAH was determined in blood and urine by the method of Bratton and Marshall (11) as modified by Smith and associates (12). Blood urea nitrogen was analyzed by the method of Van Slyke (13). Total serum proteins were determined by the micro-Kjeldahl method (14) on 12 of 20 Y group subjects, 15 of 20 M group subjects, and 11 of 14 O group subjects. Hematocrits were determined by the Wintrobe method on the control blood samples drawn from each subject.

*Analysis of data.* Clearances of inulin and PAH were corrected to 1.73 m.$^2$ of body surface. Renal afferent and efferent hydraulic resistances were calculated according to the formulae of Lamport (15) and were expressed as $10^3$ mm. Hg (min./cc.) (body surface m.$^2$/1.73 m.$^2$).

Mean blood pressure was taken as equal to the diastolic pressure plus one-half the pulse pressure. The mean serum protein values for the appropriate age group were used for the calculation of resistances in a few subjects where individual serum protein values were not available and an albumin globulin ratio of 2.2 was assumed for all subjects.

For each hemodynamic measure mean curves of the pyrogen reaction were constructed for each of the three age groups. In addition, to evaluate the significance of differences between age groups, individual response indices were computed as the difference between the baseline (average of the first two periods) and the peak response (average of the two consecutive periods which gave a maximum or minimum for the particular measurement). For the analysis of blood pressure responses, the average of periods X and XI was used instead of the individual maximum responses, because of qualitative differences in the blood pressure responses of the three age groups. The mean differences between age groups in the baseline values and response indices were evaluated for significance by the t test. Only t scores at the 1 per cent level of probability are reported as significant.

The rate of increase in $C_{PAH}$ was estimated as the slope of a straight line drawn by inspection through the points which included the period of rising $C_{PAH}$. The value for the individual was based on the average of slopes determined independently by two of the authors. The correlation between the two determinations was + 0.98.

## RESULTS

*Glomerular filtration rate* ($C_{IN}$). The mean baseline values for the $C_{IN}$ in the Y, M, and O groups were 110.4, 88.9, and 62.4 cc./min., respectively (Table I). The course of the mean $C_{IN}$ was similar through the reaction in all three age groups in that there was neither a significant increase nor decrease present (Figure 1). Subjects occasionally showed a rise or fall in $C_{IN}$ which bore no relationship to the degree of their increase in $C_{PAH}$.

*Effective renal plasma flow* ($C_{PAH}$). The mean baseline values for the Y, M, and O groups were 603, 449, and 277 cc./min., respectively (Table I). Baseline $C_{PAH}$ was unrelated to the presence or absence of any of the observed clinical characteristics. Thus, the mean baseline $C_{PAH}$ for subjects with retinal arteriosclerosis was not significantly different from the mean for those in the same age group with no retinal arteriosclerosis. The same was true for subjects with and without peripheral arteriosclerosis, elevation of systolic blood pressure, electrocardiographic abnormalities, or any combination of the observed clinical characteristics.

The group response curves are shown in Figure 1 for the three age groups. In this figure the simultaneity of time of onset, and the rate of $C_{PAH}$ increase are shown. The mean absolute increases in the clearance of PAH for the Y, M, and O groups were 416, 368, and 240 cc./min., respectively. Expressed as per cent of baseline value, the rise in $C_{PAH}$ for the Y, M, and O groups was 71, 86, and 91 per cent, respectively (Table I).

In a further attempt to characterize the increase in $C_{PAH}$ of the different age groups, a slope for the rate of reaction of each individual was obtained as an expression of rate of response per unit of time. The values obtained for the Y, M, and O groups were 5.9, 4.7, and 3.4 cc./min., respectively (Table I). Expressed as per cent of the baseline value, the rise in $C_{PAH}$ was 1.02, 1.10, and 1.35 per cent for the Y, M, and O groups, respectively.

TABLE I

*Age differences in the average hemodynamic responses to pyrogen*

| Measurement | Age groups |  |  | Significance of differences* |  |  |
|---|---|---|---|---|---|---|
|  | Y 20–49 yrs. | M 50–69 yrs. | O 70–85 yrs. | Y–M | M–O | Y–O |
| $C_{PAH}$ (cc./1.73 m.²/min.) |  |  |  |  |  |  |
| Baseline | 603 | 449 | 277 | S | S | S |
| Maximum | 1019 | 817 | 517 | S | S | S |
| Δ | 416 | 368 | 240 | S | S | S |
| Per cent increase | 71 | 86 | 91 | NS | NS | NS |
| $C_{PAH}$ rise/min. | 5.9 | 4.7 | 3.4 | NS | PS | S |
| $C_{IN}$ (cc./1.73 m.²/min.) |  |  |  |  |  |  |
| Baseline | 110.4 | 88.9 | 62.4 | S | S | S |
| FF ($C_{IN}/C_{PAH}$) |  |  |  |  |  |  |
| Baseline | .189 | .206 | .232 | NS | NS | S |
| Minimum | .115 | .123 | .122 | NS | NS | NS |
| Δ | .074 | .083 | .110 | NS | NS | S |
| Pulse rate (beats/min.) |  |  |  |  |  |  |
| Baseline | 67 | 65 | 60 | NS | PS | S |
| Maximum | 85 | 80 | 76 | NS | NS | PS |
| Δ | 18 | 15 | 16 | NS | NS | NS |
| Systolic blood pressure (mm. Hg) |  |  |  |  |  |  |
| Baseline | 116 | 124 | 136 | PS | NS | S |
| Periods X and XI | 114 | 110 | 121 | NS | NS | NS |
| Δ | 2 | 14 | 15 | S | NS | S |
| Diastolic blood pressure (mm. Hg) |  |  |  |  |  |  |
| Baseline | 77 | 77 | 75 | NS | NS | NS |
| Periods X and XI | 67 | 63 | 64 | NS | NS | NS |
| Δ | 10 | 14 | 11 | NS | NS | NS |
| Pulse pressure (mm. Hg) |  |  |  |  |  |  |
| Baseline | 39 | 47 | 61 | PS | S | S |
| Periods X and XI | 47 | 47 | 57 | NS | PS | NS |
| Δ | −8 | 0 | 4 | S | NS | S |
| $R_a$ ($10^3$ mm. Hg) (min./cc.) (body surface m.²/1.73 m.²) |  |  |  |  |  |  |
| Baseline | 6.7 | 15.5 | 31.5 | S | S | S |
| Minimum | 3.6 | 5.7 | 13.6 | S | S | S |
| Δ | 3.1 | 9.8 | 17.9 | S | S | S |
| $R_e$ ($10^3$ mm. Hg) (min./cc.) (body surface m.²/1.73 m.²) |  |  |  |  |  |  |
| Baseline | 4.5 | 8.2 | 13.7 | S | S | S |
| Minimum | 2.0 | 3.4 | 5.2 | S | S | S |
| Δ | 2.5 | 4.8 | 8.5 | S | S | S |

* S = Significant ($P < .01$) PS = Probably Significant ($P = .05$ to $.01$) NS = Not Significant ($P > .05$)

The differences in per cent increments for the three age groups were not statistically significant.

Thirteen subjects exhibited temperature rises of 1° to 3° F., and two subjects developed shaking chills of 20 minutes duration. In none of these subjects was there a significant departure of the hemodynamic response from that of the rest of the group.

*Filtration fraction* (FF). The mean baseline FF values for the Y, M, and O age groups were 0.189, 0.206, and 0.232, respectively (Table I). The absolute fall in FF was greater in the O group (0.110) than in the M (0.083) and Y groups (0.074). At the height of the reaction the differences observed in the baseline values between age groups had disappeared (Figure 1 and Table I).

*Pulse rate.* The mean baseline pulse rates in the Y, M, and O groups were 67, 65, and 60, respectively (Table I). The time of onset and the absolute magnitude of pulse rate acceleration were the same for the three age groups (Figure 2 and Table I).

*Blood pressures.* The mean baseline systolic values for the Y, M, and O groups were 116, 124, and 136 mm. Hg, respectively (Table I). The M and O groups showed a fall in pressure while the Y group remained constant throughout the reaction (Figure 2). In periods X and XI the residual differences between the age groups are not significant.

The mean baseline diastolic pressures were approximately the same (75–77 mm. Hg) for the

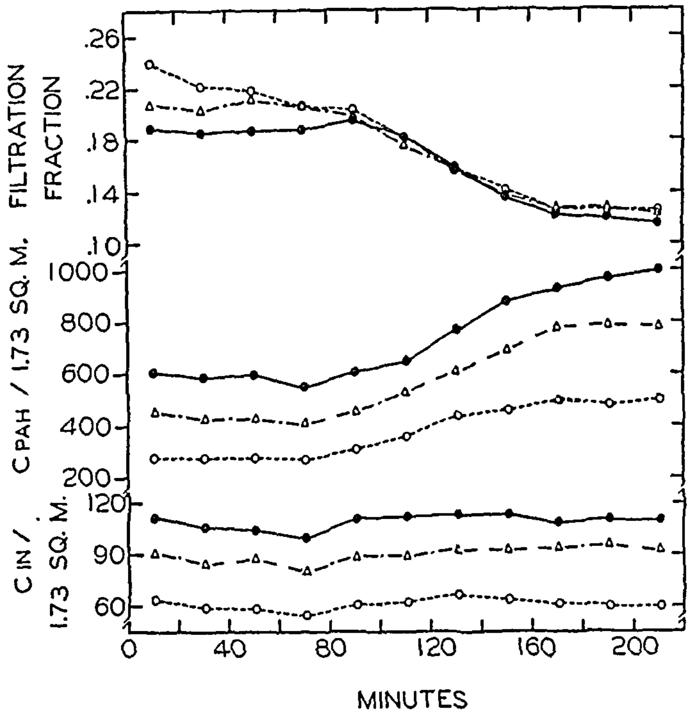

Fig. 1. Changes in $C_{IN}$, $C_{PAH}$, and FF during Pyrogen Reaction

Fifty million killed typhoid organisms were injected intravenously at 0 time.

○-----○ Mean value for 14 subjects in O group (70–85 yrs.)

△-----△ Mean value for 20 subjects in M group (50–69 yrs.)

●——● Mean value for 20 subjects in Y group (20–49 yrs.)

different age groups. This is in part due to the setting of an upper limit of diastolic pressure by the criteria of subject selection. The average fall in diastolic pressure (Figure 2) was the same for all age groups.

A rise in pulse pressure occurred in the Y group (Figure 2 and Table I) as a result of an unchanged systolic pressure and a drop in diastolic pressure. The M group showed a commensurate fall in systolic and diastolic pressures with a resultant stable pulse pressure. The O group manifested a fall in pulse pressure due to a greater fall in systolic than in diastolic pressure. There was no demonstrable relationship between the magnitude of pulse rate or pulse pressure change, or their product, and the magnitude of the $C_{PAH}$ response for the individuals in any age group.

*Serum protein and hematocrit.* Significant differences in serum protein values were observed among the three age groups. For serum protein the average values were 7.00, 6.50, and 6.10 g./100 cc. for the Y, M, and O groups, respectively. The average hematocrit values for the M and O groups were significantly lower than that for the Y group (Y = 0.44; M = 0.39; 0 = 0.39).

*Renal afferent resistance* ($R_a$). The mean baseline values for $R_a$ were 6.7, 15.5, and 31.5 $10^3$ mm. Hg (min./cc.) (body surface m.$^2$/1.73 m.$^2$), in the Y, M, and O groups, respectively (Table I). The fall in $R_a$ occurred in all three groups (Figure 3) and was for the O group 17.9, for the M group 9.8, and for the Y group 3.1 $10^3$ mm. Hg (min./cc.) (body surface m.$^2$/1.73 m.$^2$). Because of the mathematical relationships of the formula used to calculate $R_a$, the fall in these values was determined largely by the changes in mean pressure.

*Renal efferent resistance* ($R_e$). The mean baseline $R_e$ values were higher in the O group than in the M and Y groups; the values were 13.7, 8.2, and 4.5 $10^3$ mm. Hg (min./cc.) (body surface m.$^2$/1.73 m.$^2$) respectively (Table I). At the height of the pyrogen reaction, the divergence in values was

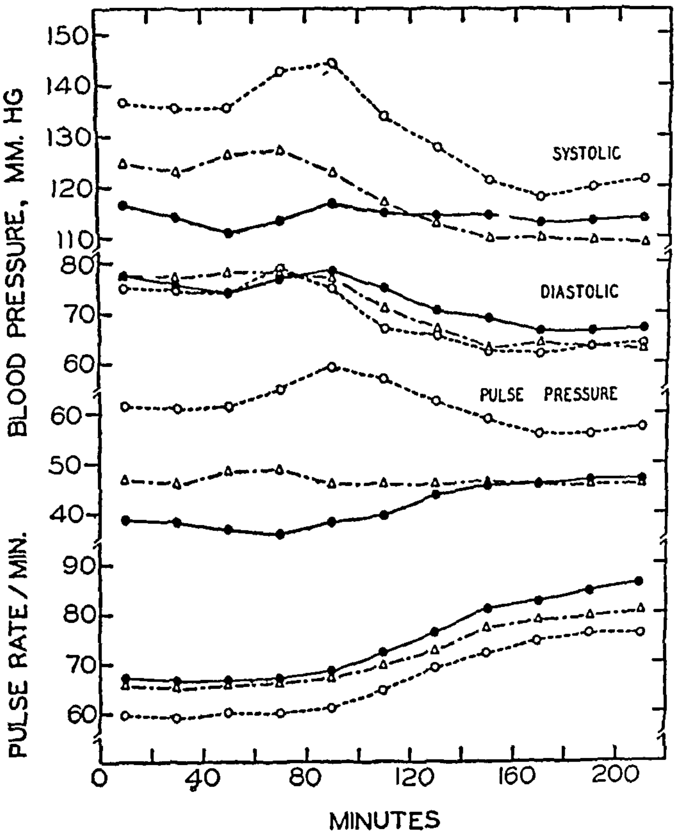

Fig. 2. Changes in Pulse Rate, Systolic, Diastolic, and Pulse Pressure during Pyrogen Reaction

Fifty million killed typhoid organisms were injected intravenously at 0 time.

○-----○ Mean value for 14 subjects in O group (70–85 yrs.)

△-----△ Mean value for 20 subjects in M group (50–69 yrs.)

●——● Mean value for 20 subjects in Y group (20–49 yrs.)

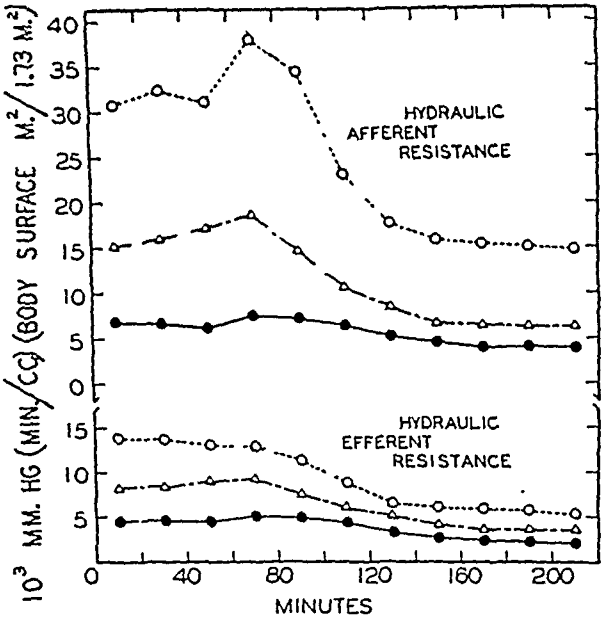

FIG. 3. CHANGES IN AFFERENT AND EFFERENT RENAL HYDRAULIC RESISTANCES DURING THE PYROGEN REACTION

Fifty million killed typhoid organisms were injected intravenously at 0 time.

○-----○ Mean value for 14 subjects in O group (70–85 yrs.)

△-----△ Mean value for 20 subjects in M group (50–69 yrs.)

●——● Mean value for 20 subjects in Y group (20–49 yrs.)

less (Figure 3); although, the $R_e$ value for the O group remained significantly higher than that of the Y and M groups.

## DISCUSSION

The decrease in the baseline $C_{IN}$ and $C_{PAH}$ in the older age groups confirms results previously reported from this laboratory (2) and are consistent with the qualitative (4, 6, 7), semi-quantitative (5), and quantitative (16) anatomic descriptions of the senile kidney. The progressively smaller absolute changes in renal plasma flow ($C_{PAH}$) in the older subjects following pyrogen are also consistent with a progressive decrease in the renal parenchyma of the aged. On the other hand, the time of onset of the response, and the percentage increase in renal plasma flow were similar in the different age groups. Consequently, it must be concluded that the responsiveness to pyrogen of the vascular elements remaining in the aged kidney is not qualitatively different from that of the young kidney.

Since direct evidence of structural changes in the renal vessels could not be obtained, indirect evidence was sought in clinical estimates of the degree of retinal, peripheral, and central arteriosclerosis present in the subjects tested. It is of interest that no correlation was found between the estimates of the degree of arteriosclerosis in these regions and the decrement in renal function within a given age group.

The results of this study also confirm the previously reported increase in FF with age (2). Since there is no reduction in the tubular extraction of PAH in the aged kidney (17), a reasonable explanation of the increased FF found in the O group is an increase in the efferent arteriolar resistance. The decrease in $C_{IN}$ and $C_{PAH}$ with age may be explained on the basis of a decrease in the number of functioning nephrons, but the observed increase in FF cannot be accounted for simply on this basis. Some change in the renal vessels beyond the point of glomerular filtration equilibrium must be postulated to account for this phenomenon. Such alteration might be present in the efferent arterioles either as structural changes, such as sclerosis, or increased vasoconstriction, or both. The fact that the FF approaches the same level in all age groups following pyrogen administration indicates that the differences are not entirely structural in nature and that the pyrogen has eliminated the inequalities of a physiologic mechanism which maintained the resting efferent arteriolar tonus at a higher level in the O than the Y group.

The calculations of renal resistances indicate an increase in both $R_a$ and $R_e$ in the resting state with age. During the response to pyrogen, both values diminish in subjects of all ages, so that at the height of the response the age differences are reduced but not entirely eliminated. The fall in $R_a$ is striking in the old group as compared to that in the young. The absolute and per cent decrement observed in $R_e$ indicates a greater degree of reversible resistance of the efferent renal arterioles in the old than in the young subjects. From the relationships between pressure and flow the presence of an initially increased $R_e$ and the ability of the efferent arterioles of the aged kidney to dilate may be inferred.

## SUMMARY

1. The renal hemodynamic response to a standard dosage of pyrogen was measured in 54 sub-

jects divided into three age groups: 20–49 years, 50–69 years, and 70–85 years.

2. The baseline $C_{IN}$ declined progressively with advancing age. No significant change in $C_{IN}$ occurred during the course of the pyrogen reaction in any age group.

3. The mean baseline $C_{PAH}$ declined progressively with advancing age. During the pyrogen reaction, the proportionate increase in $C_{PAH}$ for all age groups was approximately the same.

4. Although the baseline FF for the O group was significantly higher than the Y group, the minimum FF reached in all three groups was essentially the same.

5. The systolic blood pressure was lowest in the Y group, and did not change throughout the reaction. The systolic pressure fell significantly in the M and O groups during the reaction.

6. The diastolic blood pressure started at the same level and fell to the same degree during the reaction in all groups.

7. The baseline $R_a$ was higher, and the fall in $R_a$ during the reaction was greater in the older age groups than in the young group.

8. The efferent resistance ($R_e$) was significantly higher in each older group than in the Y group and in general paralleled the changes in FF following the administration of pyrogen. However, the minimum $R_e$ attained by the O group remained significantly higher than the minimum $R_e$ attained by the Y group.

## CONCLUSION

It has been demonstrated that the administration of pyrogen is followed by a uniform percentage increase in renal blood flow in all age groups. Since filtration fraction fell to similar values in all age groups it is inferred that the renal arterioles are capable of dilating in the aged kidney. It is therefore concluded that the reduced renal blood flow observed in the aged is in part reversible and therefore not the result of structural changes in the renal vessels alone.

## ACKNOWLEDGMENTS

We are indebted to Dr. John Bechtold for assistance in providing patients for this study; to Dr. R. W. Haines for making ophthalmological examination of the patients; and to Dr. Milton Landowne for suggestions in the analysis and interpretation of the results. Assistance in the chemical analyses was provided by Mrs. Elsie Beard, Miss Margaret McCollum, Mr. Charles Punte, Mr. Robert Faid, Mrs. Eleanor Roach, Mr. Irving Jones, Mr. Arthur Dinan, Mr. Millard Starnes, and Mr. Gilbert Bellistri.

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# URINARY EXCRETION OF CHOLINE METABOLITES FOLLOWING CHOLINE ADMINISTRATION IN NORMALS AND PATIENTS WITH HEPATOBILIARY DISEASES[1]

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(Submitted for publication November 14, 1950; accepted, March 6, 1951)

Choline has been recommended for the treatment of hepatic diseases, especially those of nutritional origin, and for the prevention of arteriosclerosis. Little is known of its fate after oral or intravenous administration. A recent Spanish report (1) that patients with liver diseases and dogs with experimental hepatic damage excrete in the urine considerably more than normal of a substance precipitated by Reinecke salt (and considered as choline by the authors) after large doses of choline given orally, suggested that the utilization of choline might be altered in hepatobiliary disease. In addition, studies with radiophosphorous (2) indicate a reduced rate of phospholipid formation in the presence of hepatocellular damage and an increased rate in biliary obstruction, suggesting that less choline might appear in the urine than normal in obstructive jaundice and more than normal in liver cell damage.

To test these hypotheses and to investigate the fate of choline after its oral administration *1*) the identity of the reineckate precipitated from the urine was determined, and *2*) the influence upon its excretion of physiologic conditions as well as hepatobiliary diseases was studied.

## MATERIAL

Four normal subjects and 22 patients with various hepatobiliary diseases were given on separate occasions single doses of choline bicarbonate[3] equivalent to 2–8 grams of choline base (230–920 mg. choline N). Their diets were not controlled. Urine samples, collected at various intervals, were preserved with 25 ml. of concentrated hydrochloric acid (diluted with equal parts of water) per liter of urine.

## EXPERIMENTAL

### *Chemical identification of the reineckate obtained from urines after choline administration and procedures applied for quantitative determination of the metabolites in the urine*

*Reagents used:*

*1*) Three per cent solution of Reinecke salt, freshly prepared and filtered.
*2*) Aqueous saturated solution of sodium hydroxide.
*3*) Aqueous saturated solution of potassium carbonate.
*4*) Folin and Ciocalteu reagent (3), diluted with three volumes of water before use.
*5*) Ten per cent aqueous solution of anhydrous sodium carbonate.
*6*) Choline reineckate: to 40 ml. of 1 per cent choline chloride 20 ml. of the Reinecke salt solution are added. After centrifugation the precipitate is washed three times with 20 ml. portions of water. The crystals are then dried at 37° C. and stored. Immediately before use a saturated solution is freshly prepared by adding some of the choline reineckate to 0.1 normal sodium hydroxide, shaking and filtering.
*7*) Acetone, Norit A, Devarda's alloy, octyl alcohol, concentrated and 0.5 normal hydrochloric acid, normal and 0.1 normal sodium hydroxide.

*The alkali-insoluble reineckate:* The reineckate precipitated at a pH of 6.5 from urines after administration of choline (following the method of the Spanish authors [1]), was almost completely soluble in 0.1 normal sodium hydroxide. Since choline reineckate was found to be hardly soluble in this solution, the great bulk of the precipitate was not choline reineckate. On nitrogen basis, only 0.7 mg. of choline reineckate is soluble in 100 ml. of 0.1 normal sodium hydroxide while 113 mg. of urinary acid-precipitable reineckate are soluble in this solution. To determine the traces of alkali-precipitable reineckate (choline) the following procedure was used, adapted from the method of Glick (4).

*Determination of choline:* Saturated sodium hydroxide is added to 70 ml. of urine until a blue color to thymolphthalein appears. The precipitate is removed by filtration and to an aliquot, representing 50 ml. of urine, 25 ml. of Reinecke salt solution are added. The Reinecke precipitate formed in four hours in the refrigerator is spun

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[1] Supported by a grant from the Dr. Jerome D. Solomon Memorial Research Foundation.

[2] Solomon Foundation Fellow.

[3] We wish to thank Commercial Solvents Corporation for the generous supply of choline bicarbonate syrup.

TABLE I

*Recovery of choline added to urine with the method used*

| Diagnosis | Mg. choline N added to 100 ml. | Mg. choline N found per 100 ml. | Per cent choline N recovered |
|---|---|---|---|
| Normal | 0.250 | 0.240 | 96 |
| | 0.750 | 0.710 | 93 |
| | 1.250 | 1.280 | 102 |
| Hepatitis | 0.250 | 0.220 | 88 |
| | 0.750 | 0.740 | 98 |
| | 1.250 | 1.360 | 107 |

down. It is washed with the saturated solution of choline reineckate in order to remove any co-precipitated noncholine reineckate. The final residue is dissolved in acetone containing 1 per cent glacial acetic acid. The solution is filtered if necessary and made to a noted volume. The transmittancy is read in a spectrophotometer or colorimeter at a wave length of 520 mµ. A standard curve is made with choline chloride using the same procedure. The results are calculated in mg. of choline N per ml. of the acetone reineckate solution. Choline added to urines from normal or hepatitis patients was recovered quantitatively (Table I). The amount demonstrated by this method represented in the studied cases 0.22 to 4.40 per cent of the total reineckate in the 24-hour urine and 0.27 to 1.04 per cent of the total amount of the choline N ingested.

*The alkali-soluble reineckate:* To demonstrate whether the alkali-soluble reineckate represents one of the recognized metabolites of choline such as betaine, dimethylamine or trimethylamine or its oxide, the reineckate precipitated from 10 ml. of acidified urine from normals and from patients suffering from hepatic diseases after administration of choline was dissolved in 20 ml. of 50 per cent aqueous acetone. Then 5 ml. of aqueous saturated silver sulfate solution were added (5) to remove the Reinecke ion. In the filtrate, the excess silver was removed with 5 ml. of normal hydrochloric acid and the water clear filtrate was evaporated under partial vacuum and under nitrogen at 30° C. The residue was dissolved in 10 ml. of methyl alcohol, filtered and evaporated as above. The final residue was dissolved in 20 ml. of water. An aliquot (10 ml.) was alkalinized with potassium carbonate and aerated for two hours at room temperature in the apparatus described by Sobel and associates (6) into 5 ml. of 0.5 normal hydrochloric acid. To this, 3 ml. of Reinecke salt solution were added and the crystalline precipitate obtained showed the microscopic appearance and melting point (275° C.) characteristic of trimethylamine reineckate. Mixing of the crystalline precipitate with trimethylamine reineckate did not alter the melting point. The melting point of the picrate was 216° C., identical with that of trimethylamine picrate and was also not altered by mixing with trimethylamine picrate.

To investigate whether trimethylamine oxide (TMAO) was also present in addition to trimethylamine (TMA) (the former not being aerated under the experimental conditions used), another aliquot of 10 ml. was acidified with hydrochloric acid to normality. It was then reduced with 0.5 gram of Devarda's alloy and placed in a water bath at 90° to 95° C. for 40 minutes. After centrifugation the supernatant was transferred to the aerating apparatus and enough saturated sodium hydroxide was added to redissolve the precipitate formed and then 3 ml. of saturated potassium carbonate were added. The mixture was aerated as before. In four experiments (two normal subjects and two patients with cirrhosis), the TMAO accounted for 90 to 97 per cent of the total reineckate, TMA for 5 to 10 per cent and choline for 0.56 to 0.77 per cent.

*Determination of total trimethylamines (TTMA) with Reinecke salt:* To 50 ml. of acid urine about 2 grams of Norit A are added and the mixture shaken and allowed

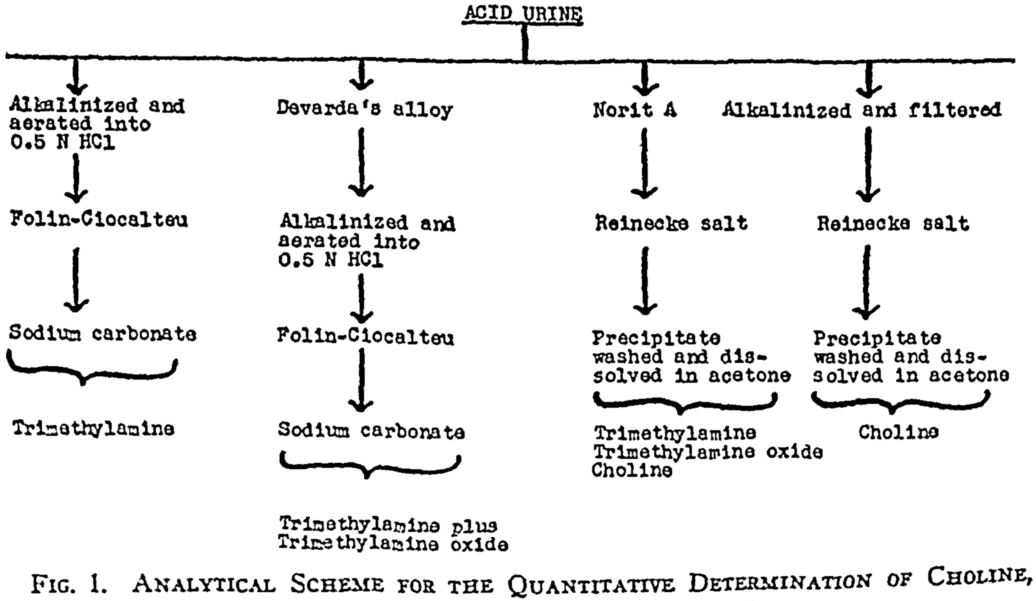

FIG. 1. ANALYTICAL SCHEME FOR THE QUANTITATIVE DETERMINATION OF CHOLINE, TRIMETHYLAMINE AND TRIMETHYLAMINE OXIDE

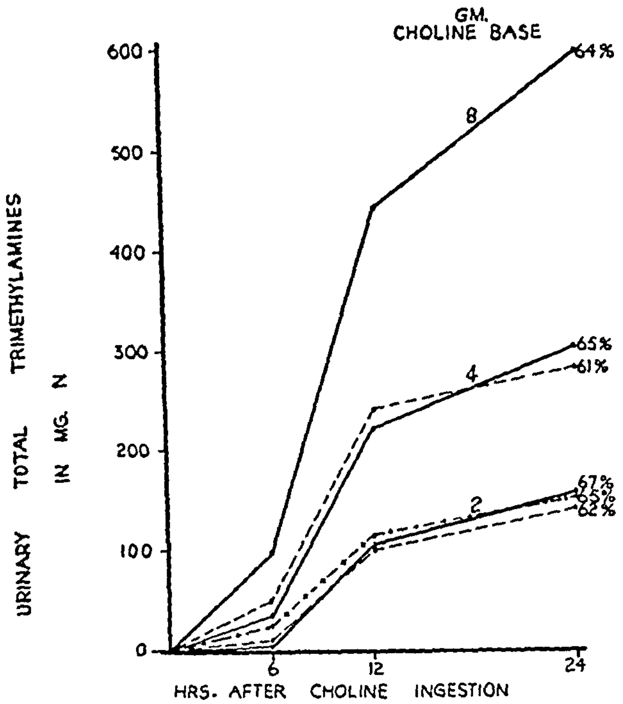

FIG. 2. URINARY EXCRETION OF TRIMETHYLAMINES BY NORMAL PERSONS AFTER ORAL ADMINISTRATION OF VARIOUS AMOUNTS OF CHOLINE

The percentage refers to the amount of trimethylamine N in relation to choline N administered. Each type of line refers to the same experimental subject.

to stand for at least 30 minutes. In some concentrated urines it is necessary to dilute the Norit filtrate. To 5 or 10 ml. of the filtrate, half a volume of the Reinecke salt solution is added. The crystalline precipitate obtained after standing at 10° C. for one hour is collected onto a fine sintered glass filter using vacuum and washed with saturated aqueous solution of "urine acid-precipitable reineckate," prepared from the same urine. The error produced by using a saturated solution of the reineckate does not exceed 20 micrograms and is, therefore, negligible. The crystals are then dissolved in acetone. The color is read in the spectrophotometer as in the determination of choline. The extinction co-efficient of the Reinecke salt of TMAO (prepared according to Dunstand and Goulding [7]), was found to be the same as that of choline, betaine and TMA chlorides when expressed as nitrogen. The very small amount of choline determined with TTMA can be ignored. The presence of betaine or other quaternary ammonium bases was excluded because the reineckate precipitable material was shown to be quantitatively removed by reduction and aeration.

*Use of Folin-Ciocalteu reagent:* The quantitative relation between TMA and TMAO was investigated by determining with the Folin-Ciocalteu reagent the TMA in the aerates from alkalinized urines with and without previous reduction. This reagent, otherwise used for the determination of phenols, gives a blue color with TMA (8) but not with ammonium, mono- or dimethylamine salts. Since the phenols of the urine are not aerated under the experimental conditions, the color reaction can be considered specific for TMA. This offers an alternative and simple method for the determination of TTMA even if present in small amounts. This method which has been used in this study for the determination in the stool cultures has subsequently been proven applicable for urine specimens.

*Method for determination of trimethylamines with the Folin-Ciocalteu reagent:* For determination of TMA, 10 ml. of urine are neutralized with saturated sodium hydroxide, followed by addition of 3 ml. of saturated potassium carbonate and 4 or 5 drops of octyl alcohol. The mixture is aerated and the TMA is collected as described before. An aliquot is neutralized with normal sodium hydroxide and made to 5 ml. with water; 2 ml. of Folin-Ciocalteu reagent and 3 ml. of the sodium carbonate solutions are added. After 15 minutes the developed color is read in a spectrophotometer at 620 mμ. A standard curve is made with pure, dried TMA hydrochloride. For determination of TTMA, 10 ml. of urine are mixed with enough concentrated hydrochloric acid to make it normal. The sample is treated with Devarda's alloy as previously described, to reduce TMAO to TMA (9). After cooling, saturated sodium hydroxide solution is added until the precipitate formed re-dissolves. Now 3 ml. of saturated po-

TABLE II

*Urinary excretion of choline and trimethylamines in normal individuals within 24 hours after administration of 2 grams choline base*

| Choline | | Total trimethylamines | | | |
|---|---|---|---|---|---|
| Mg. N | Per cent of choline N fed | Mg. N | Per cent of choline N fed | Per cent excreted in first 12 hours | (Choline/ TMA) ×100 |
| 1.20 | 0.53 | 155.0 | 67.0 | 67.5 | 0.77 |
| 0.80 | 0.35 | 142.3 | 61.8 | 70.2 | 0.56 |
| 0.90 | 0.39 | 150.0 | 65.0 | 77.0 | 0.60 |
| 0.75 | 0.33 | 138.2 | 60.0 | 65.5 | 0.54 |

tassium carbonate are added and the sample is aerated as before. The aerate is conveniently diluted and treated with the Folin-Ciocalteu reagent. TMAO is calculated from the difference between the values before and after reduction. Choline and betaine are not converted into TMA and, therefore, fail to give the reaction.

The principles of the procedures applied are illustrated in Figure 1.

### *Variations of urinary choline and TTMA excretion under physiologic conditions*

*Excretion under basal conditions:* In four normal subjects and seven patients with liver disease, no choline was demonstrated in 24-hour specimens of urine. In four normal subjects, 5.70 to 11.00 mg. of TTMA N and in 22 patients with liver disease, 5.80 to 14.99 mg. of TTMA N were found in 24-hour specimens of urine.

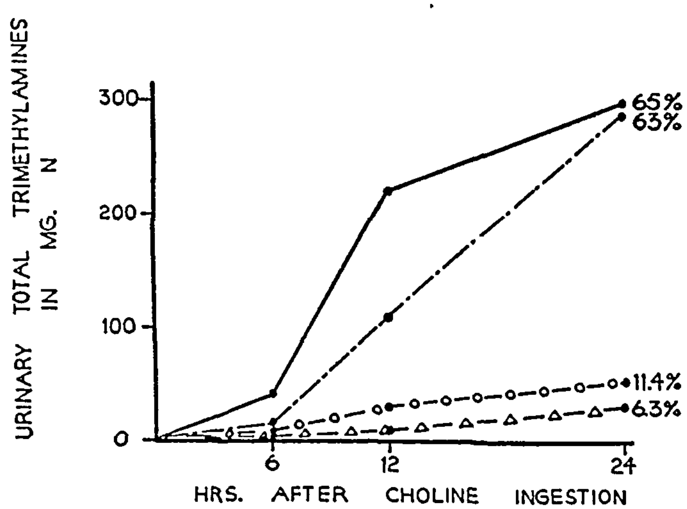

FIG. 3. URINARY EXCRETION OF TRIMETHYLAMINES AFTER ORAL ADMINISTRATION OF 4 GRAMS OF CHOLINE BASE TO A NORMAL PERSON WITHOUT SUPPLEMENT (————), AFTER FOUR DAYS TREATMENT WITH AUREOMYCIN AND SULFATHALIDINE (—O—O—), WITH SIMULTANEOUS ADMINISTRATION OF 100 GRAMS CANE SUGAR (—·—·—) AND 100 GRAMS OF STARCH (—△—△—)

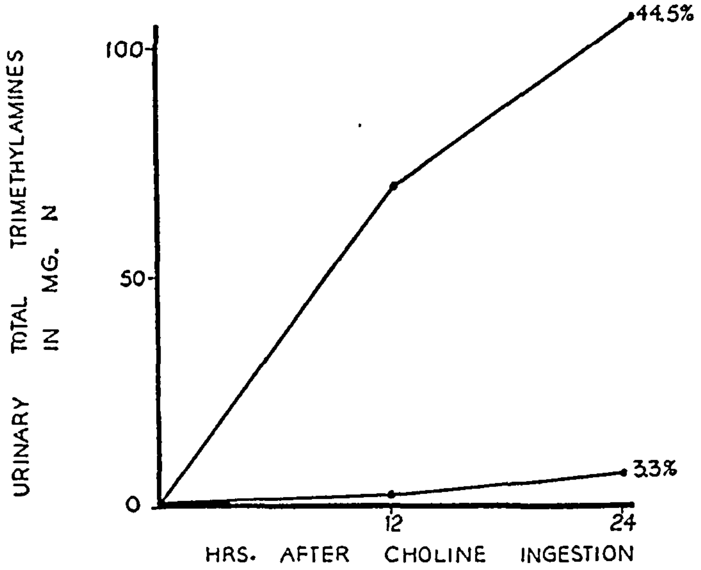

FIG. 4. URINARY EXCRETION OF TRIMETHYLAMINES BY A PATIENT WITH HEPATIC CIRRHOSIS AFTER ORAL ADMINISTRATION OF 2 GRAMS CHOLINE BASE BEFORE (UPPER LINE) AND AFTER (LOWER LINE) FOUR DAYS TREATMENT WITH AUREOMYCIN AND SULFATHALIDINE

*Response to administration of choline:* After administration of 8 grams of choline base to one normal individual, equal quantities of choline were excreted in any period observed. During the first 24 hours the total excretion was 1.40 mg. choline

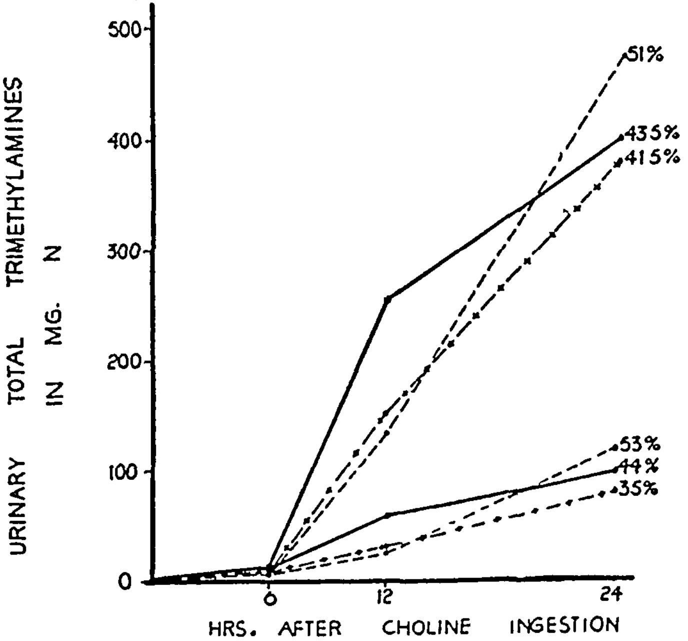

FIG. 5. URINARY EXCRETION OF TRIMETHYLAMINES BY THREE PATIENTS WITH LIVER DISEASE AFTER ADMINISTRATION OF 8 GRAMS (UPPER LINE) AND 2 GRAMS (LOWER LINE) CHOLINE BASE

Each type of line refers to the same patient.

N. Upon administration of 2 or 4 grams of choline base to four normal individuals, 0.75 to 1.60 mg. choline N were excreted in the urine. After administration of 2 to 8 grams of choline base to four normal subjects (in six experiments [Figure 2]), 60 to 67 per cent of choline N was excreted within 24 hours as TTMA of which 95 to 97 per cent was TMAO. This percentage was independent of the amount administered. Relatively little was excreted before six hours and after 12 hours, the bulk being excreted between six and 12 hours (Table II).

*Effect of ingestion of other methyl compounds:* Administration of 4 and 8 grams of betaine chloride or of 20 grams of dl-methionine to a normal individual did not cause urinary excretion of either choline or betaine and did not increase TTMA excretion.

*Influence of intestinal bacteria upon the excretion of TTMA:* Since previous investigations (10) indicated that choline is metabolized to TMA by species of the genera *Proteus, Salmonella, Eberthella* and *Shigella,* the TMA formation was determined by the aeration method in tryptose media which contained 1 per cent of choline chloride and had been inoculated with 1 drop of 1 : 1,000 saline dilution of feces. In five samples, 41 to 60 per cent of the choline present was converted to TMA in 24 hours. If 0.3 gram or less choline chloride was present in 100 ml. of tryptose medium, all choline was converted to TMA. The urinary excretion of TTMA was determined in one normal person and in two patients with cirrhosis of the liver before and after administration of 0.5 gram of aureomycin and 1 gram of sulfathalidine every four hours for four days including the day of the second choline administration. In all three instances the excretion of TTMA was greatly reduced (Figures 3 and 4 representing two of the instances). In the third one (a patient with cirrhosis) only 12-hour urine collections were available and 17.1 mg. of TTMA N instead of 78.5 mg. (without antimicrobial treatment) were excreted. Feces of the experimental subjects after treatment with aureomycin and sulfathalidine proved to be almost free of bacteria.

TABLE III

*Urinary excretion of choline and trimethylamines in patients with hepatobiliary diseases within 24 hours after administration of 2 grams of choline base*

| Diagnosis | Choline | | Total trimethylamines | | | |
|---|---|---|---|---|---|---|
| | Mg. N | Per cent of choline N fed | Mg. N | Per cent of choline N fed | Per cent excreted in first *12 hours* | (Choline/TMA) ×100 |
| Obstruct. jaundice | 1.40 | 0.65 | 152.0 | 66.0 | 46.0 | 0.92 |
| Obstruct. jaundice | 1.30 | 0.56 | 77.1 | 33.6 | 41.5 | 1.70 |
| Obstruct. jaundice | 0.79 | 0.34 | 140.0 | 60.5 | 67.0 | 0.56 |
| Infect. hepatitis | 2.38 | 1.04 | 61.0 | 26.5 | 36.0 | 3.80 |
| Infect. hepatitis | 1.18 | 0.51 | 106.0 | 46.0 | 55.0 | 1.12 |
| Infect. hepatitis | 0.75 | 0.35 | 148.1 | 64.0 | 31.0 | 0.51 |
| Infect. hepatitis | 0.62 | 0.27 | 101.4 | 44.2 | 9.2 | 0.61 |
| Infect. hepatitis | 1.90 | 0.83 | 91.7 | 39.8 | 13.2 | 2.08 |
| Chronic hepatitis | 0.31 | 0.13 | 144.1 | 63.0 | 24.7 | 0.22 |
| Chronic hepatitis | 0.59 | 0.26 | 122.4 | 53.0 | 20.3 | 0.48 |
| Cirrhosis | — | — | 106.8 | 45.5 | 70.0 | — |
| Cirrhosis | 0.70 | 0.30 | 87.9 | 38.2 | 33.1 | 0.79 |
| Cirrhosis | 0.77 | 0.34 | 80.2 | 35.0 | 34.2 | 0.96 |
| Cirrhosis with jaundice | 1.10 | 0.48 | 28.6 | 12.4 | 42.0 | 3.85 |
| Cirrhosis with jaundice | 1.02 | 0.44 | 25.0 | 10.9 | 72.0 | 4.00 |
| Cirrhosis with jaundice | — | — | 80.5 | 35.0 | 38.0 | — |
| Cirrhosis with ascites | 0.65 | 0.28 | 101.0 | 44.0 | 29.5 | 0.64 |
| Cirrhosis with jaundice and ascites | 1.35 | 0.59 | 82.5 | 35.8 | 61.0 | 1.64 |
| Cirrhosis with jaundice and ascites | 1.15 | 0.65 | 26.2 | 11.4 | 61.0 | 4.40 |
| Cirrhosis with jaundice and ascites | — | — | 25.6 | 11.2 | 39.0 | — |
| Cirrhosis with jaundice and ascites | 1.02 | 0.44 | 82.1 | 35.7 | 39.4 | 1.22 |
| Cirrhosis with jaundice and ascites | 0.85 | 0.37 | 49.5 | 21.5 | 18.2 | 1.73 |

It has been shown that glucose inhibits the conversion of choline to TMA by bacteria (10). Tryptose-choline media to which 1 per cent of glucose was added, failed to reveal upon inoculation with fecal material, any TMA formation. One normal subject who received simultaneously with 4 grams of choline base, 100 grams of cane sugar, excreted in the first 12 hours half of the TMA excreted previously without sugar adminisrtation; after 24 hours the excretion did not differ from the previous test (Figure 3).

Administration of 100 grams of starch simultaneously with choline decreased the TTMA excretion in one normal person (after ingestion of 4 grams of choline base) to one-tenth, even after 24 hours (Figure 3). In a second instance (which is not graphically presented), the TTMA excretion after administration of 2 grams of choline base decreased following starch intake from 61 to 17.4 per cent of the choline N given. The urinary choline excretion did not increase.

### *Excretion of choline and TTMA in patients with liver diseases*

In patients with liver diseases the urinary excretion of choline after administration of 2 grams of choline base was not higher than in normals. In the three patients who received 8 grams of choline base, the amount of choline excreted was higher (between 2.6 and 3.4 mg. choline N) but did not exceed one-third of 1 per cent of the total amount given.

After administration of 2 grams of choline base, two out of three patients with obstructive jaundice had a normal excretion of TTMA while the third, who died a few days later, excreted considerably less. Of seven patients with infectious hepatitis, five excreted considerably less TTMA than normal. In all 10 cases of cirrhosis investigated (two studied twice), the excretion was markedly decreased; in the two instances studied on two occasions, almost identical results were obtained. Moreover, in almost all patients with hepatic disease, the excretion was delayed in contrast to the normals. In the latter, the bulk of TTMA was excreted in the first 12 hours after the intake of choline while in the patients it was excreted between 12 and 24 hours. This delay was apparent even if the total amount excreted per 24 hours was not significantly decreased (Table III).

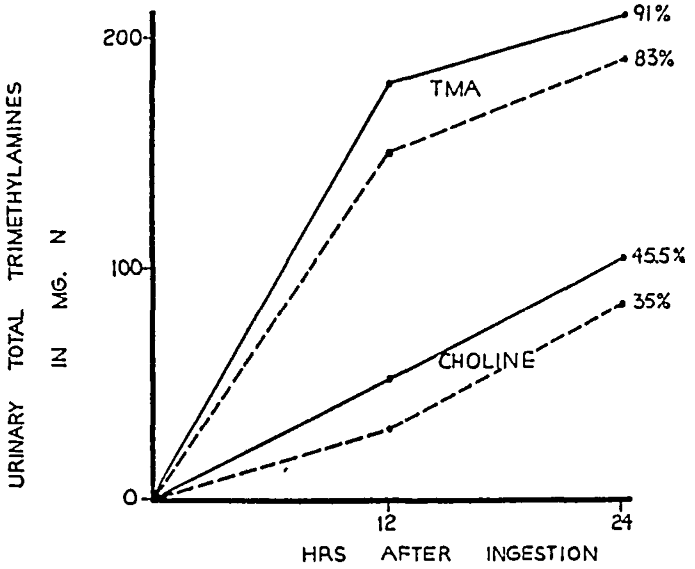

FIG. 6. URINARY EXCRETION OF TRIMETHYLAMINES AFTER ADMINISTRATION OF CHOLINE BICARBONATE AND TRIMETHYLAMINE HYDROCHLORIDE (BOTH IN AMOUNTS OF 230 MG. N) BY TWO PATIENTS WITH HEPATIC CIRRHOSIS

Each type of line refers to the same patient.

In three patients with liver disease, the percentage of TTMA N excreted in relation to the amount of choline N ingested was not significantly changed when 8 grams instead of 2 grams of choline base were given (Figure 5). Two patients with liver disease who previously had delayed and reduced TTMA excretion after choline administration and who ingested TMA hydrochloride in amounts equivalent to 230 mg. N excreted it rapidly and almost completely (Figure 6).

## DISCUSSION

In contradiction to the previously cited report on urinary choline excretion (1), this substance is excreted in the urine in insignificant amounts by normal subjects and patients with liver diseases with or without administration of large doses of choline. With the method used, the excretion rarely exceeded one-third of 1 per cent of the administered dose. Moreover, reasonable doubt exists as to whether the very small precipitate represents choline and the figures given are maximum rather than actual amounts. The bulk of the reineckate obtained in the urine after choline administration (independent of the quantity administered) was proven to be trimethylamine (TMA). The greatest part of TMA was in the oxide form. This is in keeping with previous reports that in rats (11) and in one human (12) after choline administration, TMA and TMAO appeared in the urine.

This study indicates that, as measured by two different methods, two-thirds of the choline administered appears in the urine as TMA or its oxide. The absolute amount is astonishingly well duplicated in the same person. Since many bacteria, especially those of the *Proteus* and *Shigella* groups, are known to transform choline into TMA (10), the possibility of such a process in the intestinal tract had to be considered. It was confirmed by the observation that *in vitro* feces transform choline, to a large degree, to TMA and that suppression of the intestinal flora by antimicrobial agents greatly inhibits the urinary total trimethylamines (TTMA) excretion without influence upon the choline excretion. The conclusion is justified that two-thirds of choline administered as dietary supplement, independent of the dose given, is transformed in the intestinal tract into TMA. Information as to the lipotropic activity of the latter is not available. Neither betaine nor methionine is transformed or excreted as TTMA. The fate of the remaining one-third of choline in the body is unknown.

The concept of an extensive bacterial transformation of choline into TMA appears strange in view of the fact that choline absorption should take place in the upper part of the small intestine while significant bacterial activity starts in the large intestine. Further investigations should clarify the extent of absorption of choline as such in different species.

Glucose, which is known to suppress TMA production from choline by bacteria, inhibits *in vitro* production of TMA by feces. Therefore, the suppression of intestinal TMA formation (as measured by urinary TTMA excretion) by administration of choline together with carbohydrates was attempted. Cane sugar, which is rapidly absorbed from the intestine, delayed the TTMA excretion (and probably the bacterial transformation) without reducing the total excretion in 24 hours. In contrast, starch given with choline for the entire 24-hour period considerably depressed the bacterial transformation of choline if the urinary excretion of TTMA is a measurement.

Three possible explanations have to be considered for the observed reduction or delay of the urinary TTMA excretion after choline intake found in hepatic diseases:

(*a*) Impaired absorption of TMA in the presence of liver damage. The almost complete urinary excretion of ingested TMA even in patients with liver disease speaks against this assumption.

(*b*) Faulty urinary excretion of TTMA. The complete excretion of fed TMA in liver diseases contradicts this possibility also. The bulk of TMA is excreted as its oxide. The possibility, therefore, has to be considered that the oxidized form is more readily eliminated by the kidneys; if the liver performs this oxidation, in liver diseases this function could be at fault. *In vitro* studies (13) have indicated that liver tissue reduces TMAO to TMA; therefore, the fact that the ratio between urinary TMA and TMAO is not significantly altered in liver diseases contradicts this assumption.

(*c*) Faulty bacterial action in liver diseases. Possibly the bacterial flora of the intestine is altered in patients with hepatobiliary diseases in a quantitative and/or qualitative manner which might explain the reduced formation of TMA in these conditions.

The data so far available are too scant to permit conclusions about the therapeutic value of supplementing choline therapy with antimicrobial agents or carbohydrates or about the causes of choline deficiency in the human. The utilization of orally administered choline as measured by phospholipid turnover with $P^{32}$ (14) cannot, at present, be correlated with the urinary excretion of TTMA since the latter does not reflect the fate of absorbed choline in the body. For this purpose, studies on the urinary excretion of intravenously administered choline, which are at present under way, appear more promising.

## SUMMARY

1. A procedure was adopted for the determination of choline in the urine using its precipitation by Reinecke salt, and procedures were developed for the determination of trimethylamine and trimethylamine oxide in the urine, based on either Reinecke salt precipitation or on the Folin-Ciocalteu reaction. These procedures were applied to the urines of four normal persons and 22 patients with hepatobiliary diseases before and after oral administration of 2 to 8 grams of choline base.

2. Choline was not found in the urines of normal persons nor patients with hepatobiliary diseases under basal conditions. After oral administration of choline, very small amounts, if any, of choline were excreted, as a rule not exceeding 0.3 per cent of the amount of choline administered.

3. Under basal conditions, small amounts of trimethylamines were found in the urine. Within 24 hours about two-thirds of the choline N ingested was excreted in normal individuals as trimethylamine and its oxide.

4. Incubated stool dilutions transformed choline to trimethylamine. Inhibition of the intestinal flora by aureomycin and sulfathalidine greatly depressed the urinary trimethylamine excretion. This suggests that the urinary trimethylamines result from bacterial transformation of choline in the intestine. The greater part of therapeutically administered choline is, therefore, changed in the liver into a product without known lipotropic activity.

5. Administration of cane sugar with choline delayed urinary trimethylamine excretion, while intake of starch depressed it.

6. In liver diseases the urinary trimethylamine elimination after choline administration is delayed or decreased. Ingested trimethylamine in these patients is readily excreted.

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# THE NEPHROTIC SYNDROME IN CHILDREN. AN INTERPRETATION OF ITS CLINICAL, BIOCHEMICAL, AND RENAL HEMODYNAMIC FEATURES AS VARIATIONS OF A SINGLE TYPE OF NEPHRON DISEASE[1,2]

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(Submitted for publication September 25, 1950; accepted, March 12, 1951)

It is the purpose of this report to describe and interpret the varied renal hemodynamic patterns and associated clinical and biochemical features observed in 29 children with the nephrotic syndrome studied between January, 1949, and June, 1950.

Cases of the "nephrotic syndrome" present certain uniform and consistent clinical features (1), but their separation into two groups, "nephrosis" and the "nephrotic stage of nephritis," has been rather arbitrary and frequently difficult. Normal or supernormal renal function (2) on the one hand, and hematuria, hypertension, and azotemia (3–6) on the other, have been the classical criteria for this distinction. However, review of the clinical course of patients classified as "nephrosis" with subsequent complete remission, has shown that moderate hematuria, hypertension and azotemia were common transiently observed phenomena (7–10). Moreover, serial renal function studies have demonstrated varying degrees of functional impairment in patients clinically classified as instances of either nephrosis (11, 12) or the nephrotic stage of glomerulonephritis (13–15).

This study was undertaken in order to determine:

*1)* If measurements of renal function would assist in the early differentiation of benign nephrosis from progressive nephritis,

*2)* if such a differentiation were valid physiologically, and

*3)* if studies of renal function would help to elucidate the pathogenesis of the nephrotic syndrome. A preliminary report has been published (16).

Lacking the biopsy material which would permit morphologic description of the diseased organ, the results of our physiologic studies seem to corroborate an earlier suggestion, based on pathologic studies, that both "degenerative" lipoid nephrosis and "inflammatory" glomerulonephritis are characterized by anatomical lesions and disturbances in function which are identical in kind, but different in degree (17). The data of this report indicate that the glomerulus is a primary site of the disease process, but the syndrome is frequently characterized by reversible metabolic and hemodynamic rather than solely by irreversible anatomic damage of the nephron.

## CLINICAL MATERIAL AND PROCEDURES

### *General plan of study*

Estimations of glomerular filtration rates (GFR), renal plasma flow (RPF), and maximal tubular excretory capacity ($Tm_{PAH}$), were made in 29 nephrotic children, with more than one series of simultaneous biochemical and renal functional studies in 17 of these patients. In all, 53 estimations of renal function and biochemical status were made and correlated with clinical courses. Of the 29 patients, only two were over 6.5 years of age at the time of first admission. Three patients were over 6.5 years of age at the time initial renal function studies

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[1] These studies were supported by grants from Mead Johnson & Co. and from the National Institutes of Health, U. S. Public Health Service.

[2] Presented in part before the Society of Pediatric Research, French Lick, Ind., May 1950, and the Sixth International Congress of Pediatrics, Zurich, Switzerland, July 1950.

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TABLE I

*Clinical status of all nephrotic patients observed during interval January, 1949–June, 1950*

| Nephrotic patients | No. of patients | Males | Clinical status* | | | |
|---|---|---|---|---|---|---|
| | | | Remission | Good | Poor | Dead |
| This report | 29 | 11 | 10† | 6 | 3 | 10‡ |
| Remainder clinic group | 61 | 45 | 20 | 22 | 9 | 10 |
| Total | 90 | 56 | 30 | 28 | 12 | 20 |
| Per cent | 100 | 62 | 33 | 31 | 14 | 22 |

* Remission = No edema, normal diet, full activity, minimal to absent proteinuria for at least two months.
Good = Edema, proteinuria, hypoproteinuria, hyperlipemia, no anemia, acidosis, azotemia. Urine specific gravity 1.018 or more.
Poor = Nephrotic syndrome with anemia, acidosis, azotemia, urine specific gravity "fixed" 1.010.

† Three patients have relapsed since this report was submitted for publication.

‡ Two patients died shortly after this report was prepared for publication.

were done. All other patients were less than five years of age. Omitting two extremes of age, the mean age of the group was 3.3 years and median age 3.0 years. Comments on the physiological disturbances of electrolytes and body fluids will be the subject of another report.

Appraisal of the factors which determined the selection of the particular patients for study from our clinic group was extremely difficult. Patients usually appeared in the clinic soon after manifestations of the disease were noted. The observed group of patients, therefore, was not unduly weighted by the usual preponderance of severe or advanced cases commonly noted in hospital samples. An attempt was made to select for study children with nephrosis and both with and without hematuria and/or azotemia, and with active disease of varying duration.

The sex distribution and clinical status of the patients studied is related to that of the total group of 90 nephrotic children observed in this clinic during the same time interval (Table I). The inclusion of fewer males in the study sample may be attributed to reluctance to catheterize male children with marked edema of the genitalia.

### *Blood samples*

Serum or plasma concentrations of the test substances employed were, in the majority of instances, measured in "arterial" blood obtained from capillary puncture. An inlying venous needle with fitted stylette was frequently used in larger children for sampling. Approximately 0.5 ml. whole blood was collected in 10 cm. long, 3 mm. bore, chemically clean capillary tubes—filled by direct capillarity from finger puncture (18). A drop of dilute heparin was used to wet the bore when plasma was desired; however, a considerably better yield of serum was usually obtained from the same aliquot of non-heparinized blood. The capillary tube, when filled, was capped with flanged flexible rubber caps.[4] Twenty to 40 seconds were usually required to fill a tube. After centrifugation for 30 minutes at 2,500 rpm, the tube was scratched 1 mm. above the level of packed cells, the cap at the serum end of the tube removed, and the serum-containing portion of the tube carefully broken off and recapped. The heparinized blood hematocrit was read directly by alignment of the tube against a millimeter rule. Approximately 0.2–0.3 ml. serum was thus available per tube. Two or three microtubes obtained per period sufficed for duplicate determinations. Where simultaneous thiosulfate, inulin, and PAH clearances were done, four or five tubes were obtained per period. The relatively small yield of serum, however, necessitated micromodification of usual procedures.

### *Renal function studies*

Patients were hospitalized and confined to bed for 18 to 24 hours prior to study. Fluids were allowed *ad lib.* No food was offered during the eight hour period preceding the test. All clearance studies were begun at approximately 8:30 a.m. Using a dose based on age, premedication with morphine sulfate and atropine sulfate was employed in about two-thirds of the studies in order to reduce apprehension in uncomfortable, edematous children. In general, commonly accepted techniques with few minor modifications were employed. A single injection of thiosulfate and constant infusion of inulin and PAH were used. Urine was collected through an indwelling, multi-eyed 8–14F catheter. The bladder was washed at the end of each period with sterile distilled water and with air. Urine volumes, including wash, were recorded to the nearest 0.1 ml. Thirty minutes were allowed for equilibration after the priming injection. Consecutive 15 minute collections of urine were then made for six to ten periods. In the majority of instances, blood samples were obtained at approximately 30, 60, 90, 120, 150, and 180 minutes and midpoint values of each period determined by interpolation. It was not possible to correct for urine delay time. A control venous blood sample was obtained under oil for blank determination and analysis of various serum chemical components. All sera and urine aliquots for thiosulfate and inulin analysis were precipitated within two to four hours. Analyses were completed within 24 hours.

## METHODS

Glomerular filtration rate (GFR) was determined using a single injection of sodium thiosulfate ($Na_2S_2O_3 \cdot 5H_2O$)

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[4] Obtained from the West Company, 1117 Shakamaxon St., Philadelphia, Pa.

(19) in the initial 19 observations. In the majority of instances, simultaneous inulin and thiosulfate clearances were measured. Approximately 30 mM. (8 gm.) $Na_2S_2O_3 \cdot 5H_2O$ per square meter of surface area were administered as a freshly prepared 10–17% solution. Unpleasant reactions to relatively rapid infusions (6–10 ml./min.) of this solution were rare. Concentrations of thiosulfate in serum and urine were determined in duplicate, with micromodification for serum, according to the standard periodate titrometric method. Semilogarithmic plot of urinary thiosulfate excretion and of serum concentrations against time usually followed decremental straight lines of varying slope. Initial serum concentrations approximating 40–60 mg./100 ml. were usually obtained.

The inulin clearance determined by a constant infusion technique was measured simultaneously with thiosulfate and PAH clearances in the majority of instances.[5] Serum concentrations of inulin usually approximated 20–80 mg./100 ml., and of PAH, 1.5–4.0 mg./100 ml. Inulin in serum and urine was determined by method of Roe, Epstein, and Goldstein (21) with minor modification. The standard acid $CdSO_4$ protein precipitating solution (22) was found to give as satisfactory recoveries as the Somogyi reagents, and therefore this deproteinizing agent was used. For precipitation, serum in the microtubes was transferred by capillarity into a calibrated 0.2 ml. (normax grade) ℄ pipette and delivered with three washings into 2.0 ml. distilled water contained in a 3–4 ml., 10 mm. bore, precipitin tube. The standard $CdSO_4$ precipitation was completed using one-tenth quantities of reagents. After standing 10 minutes, the precipitate was compacted by centrifugation for 20 minutes at 2,000 rpm. Duplicate tubes were prepared for each specimen and afforded sufficient supernatant for duplicate determinations of PAH and inulin. Optical density of color for inulin and PAH was determined in a Coleman Junior spectrophotometer. Maximum absorption of the resorcinol-inulin complex occurred at 510 mμ. Standard recoveries were determined with each series of analyses and an average constant in terms of optical density was derived. The constant, rather than a standard transmission curve, was used for calculation. Little variation in constants was observed in more than 70 multiple recovery determinations.

Constant infusions of PAH following a priming dose were used to measure renal plasma flow (RPF) and maximal tubular excretory capacity (Tm) (23). PAH was diluted in 5% glucose for clearance and in distilled $H_2O$ for Tm determinations. Priming doses were diluted in distilled $H_2O$. The observed PAH clearances may be low because of the unrecognized reaction of PAH with glucose (24). In the initial renal function studies, glomerular filtration rates were determined with thiosulfate immediately prior to RPF and subsequent Tm periods. Thereafter, simultaneous inulin and PAH clearance and saturation studies were carried out. Concentrations of PAH in serum and urine were determined by micromodification of a standard method (22). At high serum concentrations, 1.0 ml. aliquots of the supernatant solution obtained after precipitation (*vide supra*) were diluted 20 times with distilled water and color was developed in 10 ml. aliquots by standard macro technique. Color was read at 540 mμ in the Coleman Junior spectrophotometer. Standard recoveries and determination of recovery constants were carried out with each series of analyses. Serum blank was determined by difference. Urine blanks were found to be negligible at the dilutions used (1:2,500–10,000).

Impairment of the renal tubules may decrease the extraction ratio and may invalidate the use of uncorrected clearances for the measurement of renal plasma flow. Renal PAH extraction data are not yet available in infants and children. Cargill has reported values for renal PAH extraction of normal adult patients and of nine glomerulonephritics with varying levels of observed PAH clearance (25*a*). An exponential extraction curve has been constructed from these data and used to "correct" observed RPF for approximate extraction in order to afford an estimate of "corrected" renal plasma flow ($RPF_c$). An assumption of this type has obvious limitations, but occasionally seemed to yield a more reasonable range of magnitude for renal plasma flow.

*Estimation of surface area*

The estimation of surface area in the persistently edematous child presents an obvious problem, and ideal weight in relation to height and age is unreliable in children whose disease is of sufficiently long duration to impair linear growth and induce chronic malnutrition with wasting. A reasonable approximation of "non-edematous weight" usually was derived by comparing the observed volume of distribution ($V_D$) of thiosulfate in the edematous patient to a presumed "normal" $V_D$ estimated from thiocyanate space regression equations for height (25*b*). SCN and $S_2O_3$ may occupy somewhat different apparent distributions in view of the protein binding of SCN (26);

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[5] A comparison of 118 simultaneous thiosulfate and inulin clearance periods indicated fairly good agreement at all clearance levels, although the quantitative significance of values obtained at apparently low levels of filtration is questionable. Occasional widely discrepant values were noted. Thiosulfate clearances were usually greater than those of inulin. The average clearance ratio $\frac{\text{thiosulfate}}{\text{inulin}}$ for all periods was 1.1. Greater discrepancies were observed with the highest clearance values. The correlation coefficient of thiosulfate versus inulin clearance was 0.88 ± 0.09. The mean discrepancy between all values for simultaneous thiosulfate versus inulin clearance was 5.5 ± 3.9 ml./min. The ratio, $\frac{\text{C thiosulfate,}}{\text{C inulin}}$ was apparently independent of serum concentration of thiosulfate, $r = 0.36$, in contrast to observations in pregnant women (20).

neither accurately measures extracellular fluid volume.[6] Occasionally such estimation of surface area (SA) was obviously inadequate. Post-diuresis weight then was used to correct previous measurements of renal function to unit values. Using the derived value for "non-edematous weight," SA was estimated either by calculation or from nomograms constructed from the standard DuBois relationship: $SA = W^{0.425} \times H^{0.725} \times 71.84$.

*Other measurements*

Serum proteins were determined by micro-Kjeldahl analysis. Serum NPN (27), gasometric $CO_2$ content (28), pH (29), and cholesterol (30), were determined by standard methods. Plasma volume determinations,[7] using Evans blue (T-1824) and a modification of an acetone extraction technique (31), were carried out in all patients immediately prior to clearance studies.

## RESULTS

### *I. Clinical observations*

A summary of the clinical features of patients studied by clearance techniques is given in Table II.

The onset of symptoms was estimated from history. Since many of the children who developed the disease were quite young, observations of growth and physical status, occasionally including blood pressure and urine examination, had often been made by the family physician prior to onset of the nephrotic syndrome. When available, such information confirmed the absence of antecedent renal disease. Since renal disease of this type in children is usually associated with marked loss of appetite, lassitude, oliguria, and personality change or irritability before edema is more than suspected, parents were apt to consult their physician with the advent of definite edema. In no instance was gross hematuria, elevated blood pressure, or cardiac failure observed by the first physician to see the patient in the early phases of the disease. Upper respiratory or other infections preceded the onset of the nephrotic syndrome in only seven of 29 patients.

All patients with the nephrotic syndrome at the time of initial observation in this clinic presented the rapid or insidious onset of edema as a chief complaint. Oliguria, marked proteinuria, absence of gross or marked microscopic hematuria, hypoproteinemia (micro-Kjeldahl), and elevation of serum cholesterol were constant features. Cardiac enlargement was not observed but variable values for blood pressure and NPN were obtained in all phases of the disease (Table II). Spontaneous diuresis, frequently observed in the first weeks or months of the disease, was usually associated with temporary improvement in the values for urine and blood constituents.

*Hematuria, azotemia and anemia*

The clinical data indicated that hypertension, microscopic hematuria, and/or slight to moderate azotemia during the early months of the disease often were unreliable indices of the functional state of the kidney or subsequent course of the disease. However, essentially hyperbolic correlation was found between elevation of the NPN and diminution of GFR (Figure 1) as previously noted (32–34). Similarly, the occurrence and extent of acidosis, as indicated by serum $CO_2$ content and pH, was found to correlate roughly with reduction of GFR (35, 36) (Figure 2). The simultaneous variation of pH, $CO_2$ content and non-$CO_2$ serum buffers in relation to GFR was expressed according

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[6] The use of $V_D$ thiosulfate for the estimation of "non-edematous weight" is indicated by the following example:

Patient CB, Male—Age 1-11/12
Edematous—Before diuresis

Height = 90.8 cm.
Initial volume distribution ($V_D$) $Na_2S_2O_3$ = 8.26 L.
Predicted normal $V_D$ for height* = 3.32 L.

Estimated volume edema (predicted decrease $V_D$) = 4.94 L.
Estimated weight edema = 4.94 × 1.02 = 5.04 Kg.
Actual weight 17.70 Kg.
Edema 5.04

Estimated non-edematous weight for predicted change in $V_D$ 12.66 Kg.

* Where $V_D$ (NaScN) = $-6.14 + 0.1042$ H for SA = $0.5 - 1.2$ $M^2$ (25*b*).

Non-edematous—After diuresis

Height = 91.5 cm.
Final volume distribution ($V_D$) $Na_2S_2O_3$ = 3.23 L.
Observed decrease ($V_D$), $8.26 - 3.23$ = 5.03 L.
Estimated weight change from observed change in $V_D$ = 5.03 × 1.02 = 5.13 Kg.
Predicted weight from actual $V_D$, $17.70 - 5.13$ = 12.57 Kg.
Observed non-edematous weight = 11.88 Kg.

Error of prediction = 0.69 Kg. 6%
Estimated ideal weight from height = 13.50 Kg.
11.88

Error of prediction = 1.62 Kg. 14%

[7] Detailed data will be the subject of another report.

TABLE II

*Clinical and laboratory data of children with the nephrotic syndrome*

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Laboratory data | | | | | | | | | | | | | Clinical data | | |
| Case number | History number | Patient Age on first admission | Duration of disease in months* | Urine† | | | Blood chemistry | | | | | Hgb *gm.%* | Edema‡ | Blood pressure | Infection | | Special therapy | Diuresis | Status Months after onset |
| | | | | Protein | RBC | Casts | NPN *mg.%* | $CO_2$ content *mEq./L.* | pH | Serum proteins *gm.%* | Cholesterol *mg.%* | | | | Before onset | After onset | | | |
| 1 | 354053 | S. J.<br>4-5/12<br>♀ | 0.4<br>1.7<br>3.1<br>4.0<br>5.5 | 0.9<br>1+<br>1.2<br>1.5<br>0 | 0.2<br>1+<br>0.2<br>0<br>0.4 | 0<br>1+<br>0.3<br>0.4<br>0 | 51–25<br>27.6<br>35.4<br>26.8<br>25.1 | 19<br>24.9<br>21.5<br>27.6<br>24.0 | 7.4<br>7.4<br>7.3<br>7.4<br>7.4 | 3.8<br>5.5<br>3.5<br>4.7<br>5.8 | 590<br>750<br>395<br>260<br>140 | 15.5<br>13.5<br>12.4<br>15.2<br>11.7 | 1+<br>1+<br>1+<br>0<br>0 | 110/70<br>102/60<br>120/70<br>104/70<br>112/80 | None | Atypical pneumonia 1.5 mo. | ACTH 4 mo.; prophylactic antibiotics | Spontaneous post pneumonia; post ACTH | Temp. remission 6 mo. |
| 2 | 355028 | C. P.<br>1-4/12<br>♀ | 0.6<br>4.5<br>5.2 | 3+<br>3+<br>± | 4–10<br>2–4<br>Rare | 4<br>4 | 63.6<br>33.1<br>25.7 | 20.6<br>20.0<br>30.5 | 7.2<br>7.2<br>7.4 | 4.0<br>4.7<br>4.9 | 680<br>615 | 9.5<br>8.1<br>9.2 | 1+<br>2+<br>0 | 110/60<br>100/60<br>114/64 | Diarrhea etiology? 0.5 mo. | None | ACTH 5 mo.; antibiotics | Initial spontaneous; post ACTH | Remission 6 mo. |
| 3 | 338957 | A. G.<br>1-3/12<br>♀ | 1.0<br>14.0 | 3+<br>2+ | 2–4<br>2–4 | 4<br>4 | 60.0<br>39.3 | 18.3<br>11.3 | 7.2<br>7.2 | 3.6<br>2.9 | 485 | 15.0<br>12.8 | 4+<br>1+ | 116/70<br>120/80 | None | None | Multiple paracenteses and transfusions | Early in course | Died. Reported uremia and cardiac failure 16 mo.§ |
| 4 | 359815 | L. A.<br>2-3/12<br>♀ | 1.0 | 3+ | 2–4 | 0–2 | 22.4 | 25.8 | 7.4 | 4.2 | 570 | 12.0 | 1+ | 110/70 | None | URI | Prophylactic antibiotics | Spontaneous 2 wks. post onset; 3–4 times since | Intermittent edema, proteinuria 3 mo. |
| 5 | 339231 | R. S.<br>6-6/12<br>♂ | 1.0<br>14.0 | 3+<br>3+ | 0–10<br>2–4 | 0–4<br>0–1 | 61.4<br>55.9 | 22.3<br>19.0 | 7.4<br>7.3 | 3.7<br>3.4 | | 13.6<br>8.0 | 4+<br>1+ | 130/98<br>120/70 | URI 0.5 mo. | None | Thoracenteses; paracenteses | None | Died.¶ Uremia 17 mo. |
| 6 | 339226 | R. M.<br>2-7/12<br>♂ | 1.5 | 3+ | 10 | 2–4 | 47.4 | | | 4.2 | | 16.0 | 2+ | 130/100 | URI 0.5 mo. | *B. coli* 5 mo.; alpha strep. septicemia 7 mo. | Thoracentesis; antibiotics | Post *B. coli* infection | Died.** Alpha streptococcus septicemia 7 mo. |
| 7 | 339937 | B. D.<br>1-8/12<br>♀ | 2.0 | 3–4+ | 0–10 | 0–2 | 105.0 | 24.3 | 7.3 | 3.7 | 445 | 13.4 | 3+ | 125/70 | Vaccinia 0.4 mo. | None | None | None | Unknown 20 mo. |
| 8 | 353096 | G. B.<br>1-10/12<br>♂ | 2.0<br>2.8<br>8.8 | 0.8<br>0.6<br>4+ | 5.0<br>1.4<br>6–8 | 1.0<br>0<br>1–2 | 48.3<br>22.1<br>52.9 | 21.7<br>23.3<br>20.4 | 7.4<br>7.3<br>7.2 | 4.1<br>5.5<br>3.2 | 800<br><br>700 | 13.4<br>10.2<br>8.8 | 4+<br>±<br>4+ | 118/88<br>98/82<br>120/90 | None | Cellulitis 5 mo. | ACTH 2.5 and 11 mo. | Post 1st ACTH only | Early renal failure 9 mo. |
| 9 | 356191 | H. M.<br>3-4/12<br>♂ | 2.5<br>5.0 | 0.5<br>3+ | 0.4<br>0 | 0<br>1–2 | 35.4<br>36.4 | 27.5<br>27.8 | 7.4<br>7.2 | 4.3<br>4.0 | 400<br>615 | 12.2<br>13.5 | 1+<br>3+ | 108/60<br>120/60 | URI 0.3 mo. | "Tonsillitis" 1 mo. | ACTH 5 mo.; paracenteses; antibiotics | Initial spontaneous 2 wks.; none since | Active disease 6 mo. |
| 10 | 339952 | J. M.<br>4-8/12<br>♀ | 4.0<br>18.0 | 3–4+<br>3–4+ | 0–2<br>0–2 | 0–1<br>0–1 | 30.0<br>31.3 | 23.8<br>16.7 | 7.5<br>7.3 | 4.8<br>3.6 | 444<br>470 | 14.0<br>9.7 | 2+<br>1–2+ | 106/90<br>94/70 | None | Recurrent cellulitis and bronchitis | Transfusions | Spontaneous 5 and 18 mo. | No edema, uremia 21 mo. |
| 11 | 338069 | F. K.<br>2-3/12<br>♂ | 4.0<br>16.0 | 3.5<br>4.6 | 0.2<br>39.6 | 0<br>1.8 | 33.1<br>91.6 | 25.3<br>13.4 | 7.4<br>7.1 | 3.5<br>3.8 | | 14.6<br>5.3 | 3+<br>1+ | 90/70<br>92/78 | None | None | Human serum albumin | Spontaneous 1 mo.; again with uremia 14 mo. | Died. Uremia 17 mo.§ |
| 12 | 359828 | J. Mn.<br>3-0/12<br>♂ | 4.0<br>4.7 | 4+<br>0.1 | 5.1<br>1.6 | 19.7<br>0.1 | 60.6<br>60.6 | 21.9<br>27.7 | 7.3<br>7.4 | 3.8<br>4.3 | 570<br>470 | 11.7<br>10.5 | 3+<br>± | 106/68<br>108/64 | URI 0.3 mo. | None | ACTH 4 mo. | Spontaneous 1 wk. and post ACTH | Remission 6 mo. |

TABLE II—*Continued*

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Laboratory data | | | | | | | | | | | | | Clinical data | | |
| | | | | Urine† | | | Blood chemistry | | | | | | | | Infection | | | | |
| Case umber | History number | Patient Age on first admission | Duration of disease in months* | Protein | RBC | Casts | NPN *mg.%* | $CO_2$ content *mEq./L.* | pH | Serum proteins *gm.%* | Cholesterol *mg.%* | Hgb *gm.%* | Edema‡ | Blood pressure | Before onset | After onset | Special therapy | Diuresis | Status Months after onset |
| 13 | 337285 | J. F.<br>3-11/12<br>♀ | 4.0<br>17.0 | 3+<br>4+ | 0–2<br>4–10 | 0–1<br>4–10 | 39.2<br>39.7 | 25.5<br>21.2 | 7.4<br>7.3 | 3.2<br>4.4 | 730 | 12.0<br>12.6 | 2+<br>0 | 100/60<br>86/48 | None | Probable peritonitis 2 times in first 18 mo. | None | Spontaneous 17 mo. | No edema, uremia 35 mo. |
| 14 | 353044 | G. C.<br>2-1/12<br>♂ | 5.0 | 0.9 | 1.1 | 0.7 | 33.1 | 24.9 | 7.4 | 3.1 | | 13.8 | 2+ | 90/60 | Severe sunburn 0.3 mo. | None | None | Spontaneous 1 and 8 mo. with remission | Remission 12 mo. |
| 15 | 342134 | R. G.<br>3-1/12<br>♂ | 6.0<br>7.5 | 4+<br>3+ | 0–2<br>2–4 | 0–1<br>4–10 | 25.9 | 24.0<br>26.4 | 7.4<br>7.2 | 3.2<br>3.6 | | 12.3<br>10.5 | 3+<br>2+ | 116/78<br>112/70 | None | Cellulitis; pneumonia; pneumococcal septicemia; terminal staph. albus septicemia | Human serum albumin; measles 7 mo.; thoracenteses, paracenteses; ACTH 15 mo. | None | Died.†† Cardiac failure 17 mo. |
| 16 | 338933 | P. N.<br>2-8/12<br>♀ | 6.0 | 4.5 | 2.3 | 0.5 | 70–23 | 24.4 | 7.4 | 3.4 | 478 | 13.8 | 3+ | 130/96 | None | Measles | Measles 3 mo. | Following measles and 2 times since | No edema, proteinuria 25 mo. |
| 17 | 350433 | R. B.<br>1-11/12<br>♂ | 7.0<br>11.0<br>12.0 | 2.2<br>1.5<br>1.5 | 4.8<br>55.0<br>0.7 | 2.0<br>1.2<br>0.1 | 24.7<br>45.8<br>29.1 | 20.2<br>14.4<br>24.8 | 7.4<br>7.2<br>7.4 | 5.0<br>3.2<br>4.2 | 460<br>304 | 10.8<br>8.4<br>10.2 | 1+<br>4+<br>± | 110/70<br>125/75<br>98/52 | None | None | ACTH 6 and 11 mo. | Post ACTH | Temporary remission 14 mo. |
| 18 | 328927 | E. D.<br>2-0/12<br>♀ | 7.0<br>7.5<br>22.0 | 3+<br>2+<br>3+ | 10+<br>4–5<br>0–3 | 0–1<br>0–1 | 67.7<br>69.7<br>98.0 | 24.0<br>24.0<br>12.9 | 7.3<br>7.3<br>7.2 | 2.9<br>3.2<br>3.2 | 730<br>800 | 12.0<br>10.2<br>6.2 | 4+<br>±<br>2+ | 110/70<br>100/60<br>110/80 | None | None | Human serum albumin; frequent paracenteses and transfusions | Post paracentesis | Died.‡‡ Uremia 30 mo. |
| 19 | 346666 | H. S.<br>3-0/12<br>♂ | 8.0<br>12.7 | 3+<br>0.4 | 0–10<br>0 | 4–10<br>2.0 | 52.8<br>33.1 | 21.2<br>21.8 | 7.3<br>7.2 | 3.8<br>3.7 | 412 | 11.0<br>10.8 | 2+<br>3+ | 100/70<br>110/80 | URI 0.5 mo. | Paracolon septicemia 11 mo.; cellulitis 12 mo. | Paracenteses, thoracenteses; ACTH 13 mo. | Post paracenteses; post ACTH | Remission 14 mo. |
| 20 | 342976 | D. G.<br>7-8/12<br>♀ | 9.0 | 2.5 | 5.3 | 6.9 | 45.8 | | | | | 14.2 | 3+ | 106/72 | None | Peritonitis; cellulitis | None | Following cellulitis | Active disease 24 mo. |
| 21 | 343049 | L. C.<br>3-1/12<br>♀ | 15.0 | 3+ | 0–2 | 0–1 | 26.8 | 24.3 | 7.4 | 5.3 | | 14.7 | 0 | 98/58 | URI 0.3 mo. | Bronchitis 5 mo.; peritonitis 9 mo. | Prophylactic antibiotics | Spontaneous 10 mo., with remission | Remission 20 mo. |
| 22 | 342905 | H. O.<br>14-6/12<br>♀ | 15.0<br>26.0 | 6.8<br>4+ | 63.4<br>4–10 | 3.9<br>0–1 | 43.7<br>26.9 | 28.0<br>25.2 | 7.4<br>7.3 | 3.1<br>2.8 | 650 | 17.5<br>10.4 | 4+<br>1+ | 158/116<br>200/128 | None‖ | None | Human serum albumin | None | Active disease 30 mo. |
| 23 | 329846 | W. L.<br>2-10/12<br>♂ | 15.5 | 3+ | 10+ | 0–1 | 116.8 | 9.0 | 7.2 | 3.9 | | 9.5 | 1+ | 110/80 | URI | Mumps 0.5 mo. Pneumococcal peritonitis 8, 10, 13 mo.; pneumonia | Human serum albumin | Post infection 8 mo. | Died.§§ Uremia 16 mo. |
| 24 | 344988 | E. G.<br>4-2/12<br>♀ | 17.0<br>30.0 | 3+<br>± | 4–10<br>0–2 | 0–1<br>0 | 37.3<br>23.4 | 21.2<br>29.5 | 7.1<br>7.4 | 3.0<br>5.4 | 756<br>540 | 10.4 | 4+<br>0 | 124/80<br>110/65 | Measles 1 mo. | Cellulitis; bronchitis | Human serum albumin | Spontaneous 2 yrs. | Remission 34 mo. |

TABLE II—*Continued*

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Laboratory data | | | | | | | | | | | | | Clinical data | | |
| | | | | Urine[†] | | | Blood chemistry | | | | | | | | Infection | | | | |
| Case number | History number | Patient Age on first admission | Duration of disease in months[*] | Protein | RBC | Casts | NPN *mg.%* | $CO_2$ content *mEq./L.* | pH | Serum proteins *gm.%* | Cholesterol *mg.%* | Hgb *gm.%* | Edema[‡] | Blood pressure | Before onset | After onset | Special therapy | Diuresis | Status Months after onset |
| 25 | 353073 | S. S.<br>4-9/12<br>♀ | 18.5<br>19.0 | 0.6<br>2.4 | 0.8<br>0.6 | 2.2<br>0.7 | 38.8<br>44.6 | 22.0<br>19.8 | 7.3<br>7.2 | 3.2<br>3.4 | 475<br>475 | 10.2 | 2+<br>3+ | 106/70<br>130/90 | None | Frequent bronchitis; probable peritonitis | Paracenteses; ACTH 17 mo. prophylactic antibiotics | Post ACTH and paracentesis with remission | Remission 24 mo. |
| 26 | 325742 | B. S.<br>3-10/12<br>♀ | 20.0 | 4+ | 0-2 | 4-10 | 152.0 | 15.2 | 7.3 | 4.7 | | 9.0 | 2+ | 118/100 | None | Measles 5 mo. | Frequent paracenteses | Minimal post measles | Died 33 mo.[§] |
| 27 | 330624 | L. N.<br>2-7/12<br>♀ | 22.0 | 3+ | 0-2 | 4-10 | 65.0 | 9.2 | 7.3 | 3.2 | | 8.5 | 1+ | 90/50 | None | Peritonitis; cellulitis | Human serum albumin; paracenteses | Post infection 9 mo.; spontaneous 22 mo. | Died. Uremia 36 mo.[§] |
| 28 | 341502 | E. M.<br>4-6/12<br>♀ | 35.0 | 3+ | 0-10 | 0-1 | 72.8 | 11.9 | 7.3 | 3.8 | 365 | 8.4 | 4+ | 120/88 | None | Uncertain | Frequent paracenteses | None | Died. Uremia, cardiac failure 37 mo.[§] |
| 29 | 269313 | A. O.<br>2-7/12<br>♀ | 64.0 | 3+ | 0-2 | 0 | 30.8 | 27.8 | 7.5 | 4.8 | | 14.8 | 0 | 104/70 | URI | Measles 13 mo.; pneumococcal septicemia 26 mo. | Measles; human serum albumin | Post measles; with albumin 13 mo.; spontaneous 53 mo. | Remission. ± proteinuria 65 mo. |

[*] Time estimated from first appearance of edema. Although many observations were available for each patient, only those coincident in time with observations of Table III are recorded herein.

[†] Urinalyses were either semiquantitative (Addis) or qualitative. Decimalized data in columns 5–7 refer to semiquantitative excretions per 12 hour period. Proteinuria expressed in grams; RBC and cast excretions as millions per 12 hours. Qualitative analyses are usually given as ranges; RBC and casts as numbers per high-powered field of centrifuged sediment.

[‡] Edema estimated on an arbitrary scale of none = 0 to extreme = 4+.

[§] No autopsy.

[‖] Anorexia, pallor and weakness for several months before edema.

[¶] Postmortem: large (approximately twice normal weight) pale kidneys. Capsule somewhat adherent with a granular surface studded with petechiae. Cortico-medullary boundaries fairly well preserved. Most glomeruli were fibrosed; viable remainder evidenced marked proliferative response. Interstitial fibroses, small round cell infiltration, tubular dilatation, and atrophy were evident.

[**] Postmortem: right adrenal medullary hemorrhage. Kidneys twice normal weight; cut surface, loss of cortico-medullary zone. Moderate to marked epithelial hypertrophy of most glomeruli with beginning crescent formation and occasional partial hyalinization. Lipoid in tubular cytoplasm with some areas of vacuolization.

[††] Postmortem: atheromatosis of aorta, and normal sized kidneys studded with petechiae. Poor cortico-medullary differentiation. Glomeruli showed all degrees of involvement; normal, increased cellularity and crescent formation to hyalinization. Hypertrophy and atrophy of tubules with some lipoid deposition and vacuolization of tubular epithelium. Arterioles showed varying degrees of intimal proliferation.

[‡‡] Postmortem: *E. coli* bacteremia, peritonitis, pneumonia. Parathyroid hyperplasia and hypoplasia of bone marrow grossly evident. Kidneys were three-fourths of normal size, pale, easily stripping capsule and poor cortico-medullary differentiation on cut surface. Almost all glomeruli showed involvement varying from round cell infiltration and slight proliferation to complete scarring and hyalinization. No active crescent formation. Tubular degeneration and atrophy with isolated dilated, hypertrophied tubules evident. Fat largely in glomerular blood vessels.

[§§] Postmortem: Parathyroid hyperplasia; slight atherosclerosis. *Staph. albus* cultured from pleural cavity; *Staph. aureus* from lungs and peritoneal cavity. Heart blood culture negative. Kidneys small and capsule slightly adherent. Small white granules on the surface. Cortical zone appeared well preserved grossly, but most glomeruli showed either hypercellularity or thickening, frequently with crescent formation. The tubules were generally atrophied with occasional necrosis of the epithelial linings.

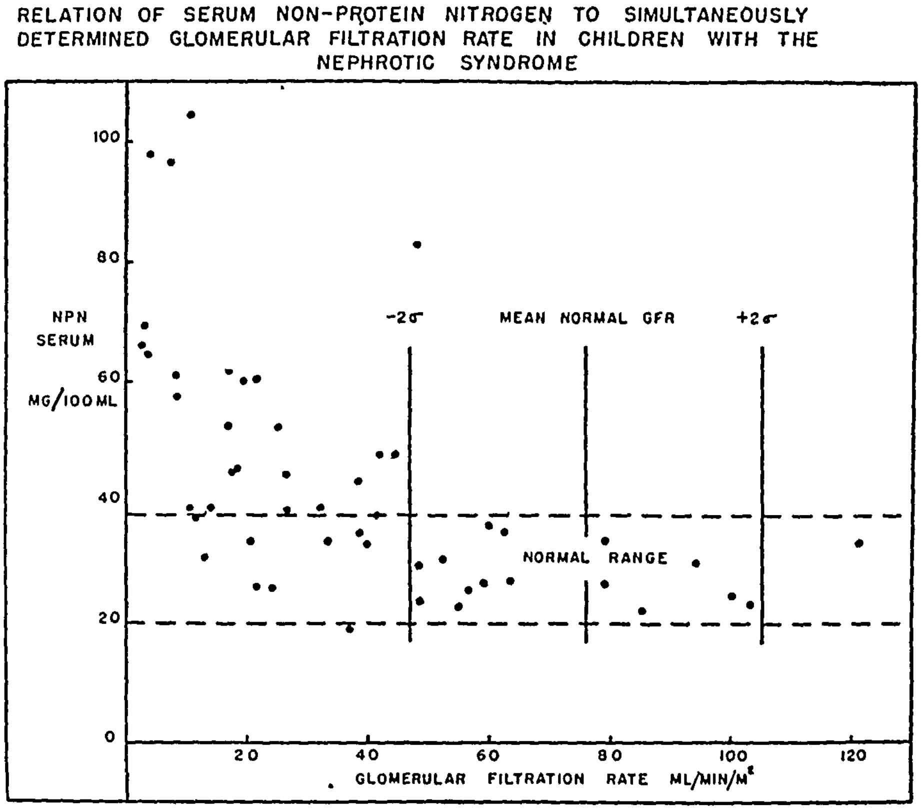
RELATION OF SERUM NON-PROTEIN NITROGEN TO SIMULTANEOUSLY DETERMINED GLOMERULAR FILTRATION RATE IN CHILDREN WITH THE NEPHROTIC SYNDROME
NPN
SERUM
MG/100 ML
100
80
60
40
20
0
-2σ
MEAN NORMAL GFR
+2σ
NORMAL RANGE
20
40
60
80
100
120
GLOMERULAR FILTRATION RATE ML/MIN/M²

Fig. 1.

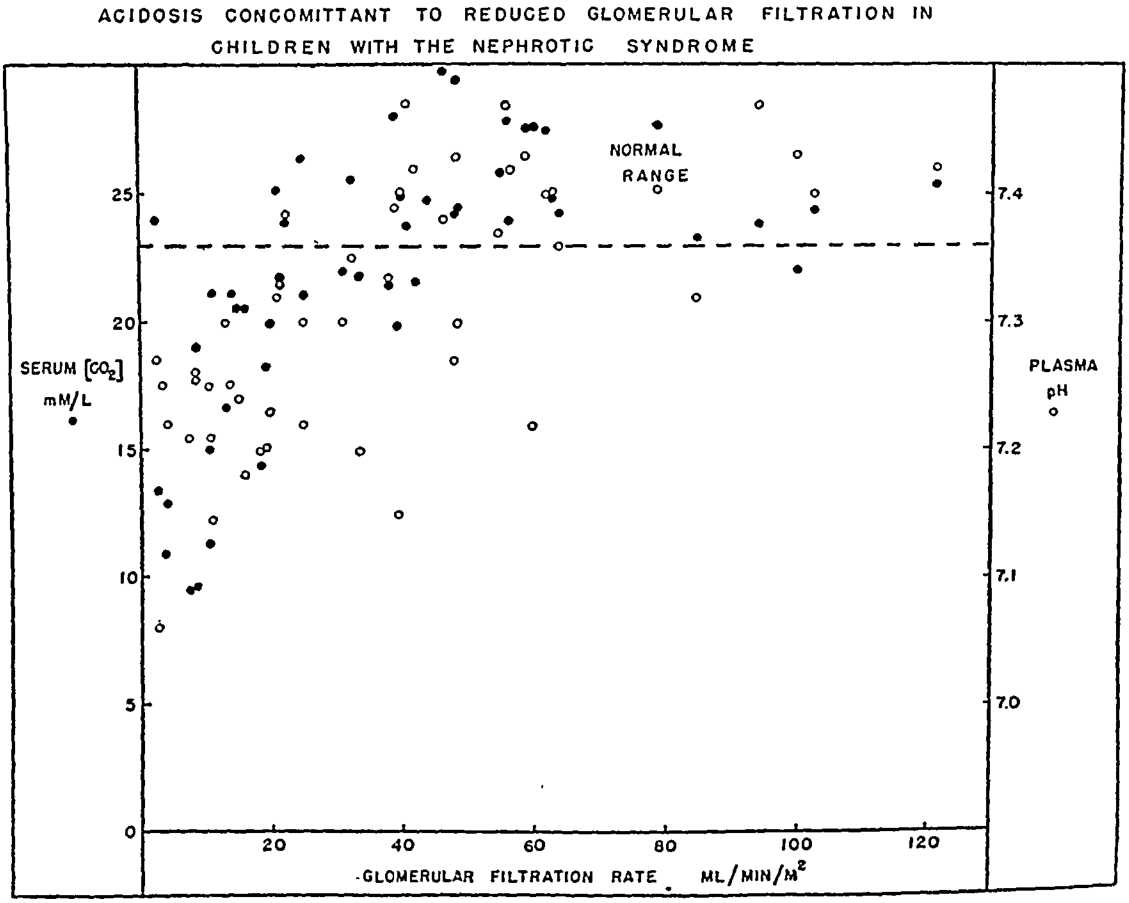
ACIDOSIS CONCOMITTANT TO REDUCED GLOMERULAR FILTRATION IN CHILDREN WITH THE NEPHROTIC SYNDROME
NORMAL
RANGE
SERUM [CO₂]
mM/L
●
PLASMA
pH
○
25
20
15
10
5
0
7.4
7.3
7.2
7.1
7.0
20
40
60
80
100
120
GLOMERULAR FILTRATION RATE ML/MIN/M²

Fig. 2.

to the buffer base concept suggested by Singer and Hastings (37) (Figure 3). In approximately half of the patients, whole blood buffer base was estimated. In these instances, the derived values were slightly different; the trend and relationships were the same. It was evident that reduction of serum buffer base was usually associated with reduced GFR; however, reduction of GFR was observed occasionally without concomitant lowering of serum buffer base. Anemia, when accompanying azotemia and acidosis, completed a triad which indicated marked limitation of renal regulation and suggested a very poor prognosis. Anemia is rare early in the nephrotic syndrome. These three variables, rather arbitrarily related to each other, have been expressed as a ratio and correlated with GFR (Figure 4). The limitations of such an expression are obvious. In most instances, reduction in the arbitrary ratio accompanied roughly proportional reduction in GFR. However, one child (No. 24),[8] with moderate reduction in the arbitrary ratio after 17 months of active disease, appeared to be in clinical remission approximately one year later although functional data suggested residual renal damage. Several children with very low ratios have died (Nos. 5, 11, 18, 23, 26, 28); the others have persistent nephrotic symptoms and ample evidence of renal insufficiency.

### *Renal function data*

The pertinent renal function data for each patient are presented in Table III and may be correlated with the clinical and biochemical observations in Table II. The validity of the "clearance" concept in the presence of nephron damage has been justifiably questioned (38–40). Some data on the response to adrenocorticotropic hormone (ACTH) therapy is included since ACTH may indirectly alter the metabolic activity of viable nephron cells (41, 42).[9] It can hardly be expected to restore destroyed, fibrosed or hyalinized tissue. Therapeutic response, therefore, affords one possible means of contrasting reversible impairment of function with irreversible destruction of parenchyma.

### *Glomerular filtration rate.*

The distribution of values for GFR in relation to the duration of the disease is depicted in Figure 5. The course of certain patients is indicated. In

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[8] Here and elsewhere, patients' numbers are as in Tables II and III.

[9] A detailed report concerning physiologic and clinical response to ACTH is in preparation.

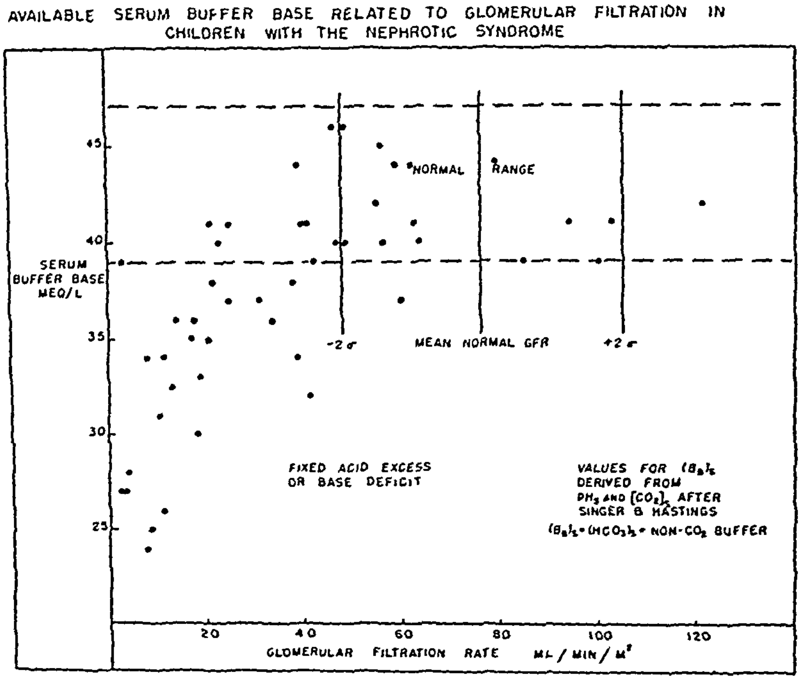
AVAILABLE SERUM BUFFER BASE RELATED TO GLOMERULAR FILTRATION IN CHILDREN WITH THE NEPHROTIC SYNDROME

FIG. 3.

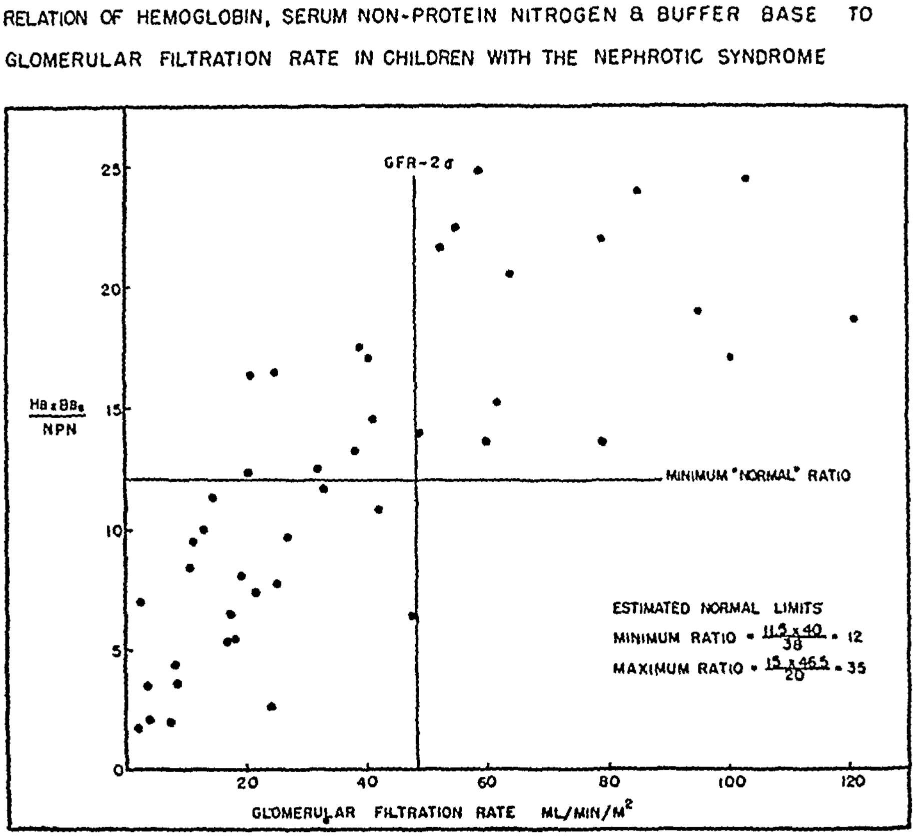
RELATION OF HEMOGLOBIN, SERUM NON-PROTEIN NITROGEN & BUFFER BASE TO GLOMERULAR FILTRATION RATE IN CHILDREN WITH THE NEPHROTIC SYNDROME
GFR-2σ
$\frac{Hb \times BB_s}{NPN}$
25
20
15
10
5
0
MINIMUM "NORMAL" RATIO
ESTIMATED NORMAL LIMITS
MINIMUM RATIO = $\frac{11.5 \times 40}{38}$ = 12
MAXIMUM RATIO = $\frac{15 \times 46.5}{20}$ = 35
20
40
60
80
100
120
GLOMERULAR FILTRATION RATE ML/MIN/M²

FIG. 4.

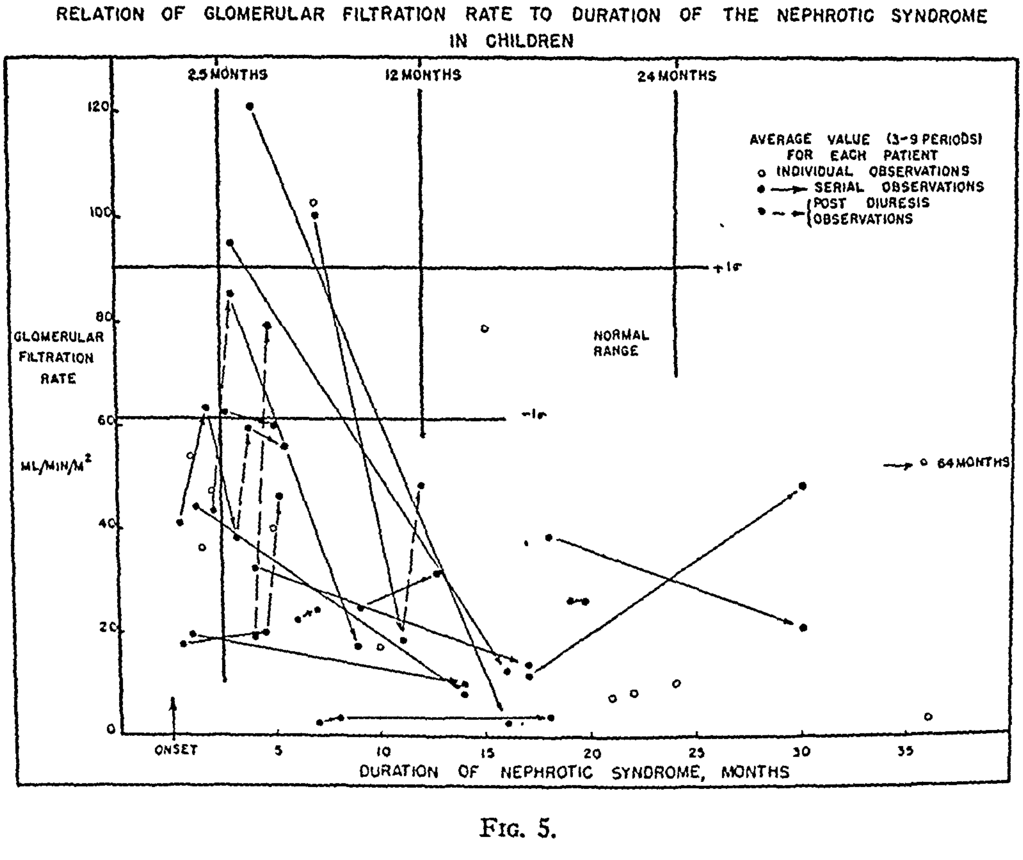
RELATION OF GLOMERULAR FILTRATION RATE TO DURATION OF THE NEPHROTIC SYNDROME IN CHILDREN
2.5 MONTHS
12 MONTHS
24 MONTHS
AVERAGE VALUE (3-9 PERIODS) FOR EACH PATIENT
○ INDIVIDUAL OBSERVATIONS
●→ SERIAL OBSERVATIONS
●- -→ POST DIURESIS OBSERVATIONS
120
100
80
60
40
20
0
GLOMERULAR FILTRATION RATE
ML/MIN/M²
+1σ
NORMAL RANGE
-1σ
→ ○ 64 MONTHS
ONSET
5
10
15
20
25
30
35
DURATION OF NEPHROTIC SYNDROME, MONTHS

FIG. 5.

TABLE III

*Renal function data of children with the nephrotic syndrome**

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Patient data | | | | | | | | Renal functions/M² (average) | | | | | | | | | | V$_D$ Thio.‡ | |
| Case number | Patient | Determination number | Age (*years*) | Height (*cm.*) | Weight (*Kg.*) | Surface area (*M²*) | Duration of disease (*months*) | GFR inulin *ml./min.* | GFR $Na_2S_2O_3$ *ml./min.* | RPF *ml./min.* | Est. extract. ratio† | RPF$_e$† *ml./min.* | TmPAH | $\frac{GFR}{RPF}$ | $\frac{GFR}{RPF_e}$† | $\frac{RPF}{TmPAH}$ | $\frac{GFR}{TmPAH}$ | Liters | λe |
| Normal values: | | 25 Children (49)§<br>1-5/12–10-1/12<br>61 Adults (22) | | SA =0.48–1.24<br>SA =1.48–1.91 | | 1.00<br>1.00 | | 76±15<br>75±12 | 74(19) | 364±69<br>403±78 | 0.89<br>0.90 | 410±70<br>448 | 42±10<br>47 | 0.21±.05<br>0.19±.02 | 0.19<br>0.17 | 9.0±3.5<br>8.6 | 2.1±0.9<br>1.7 | | |
| 1 | S. J. ♀ | 1 | 4-5/12 | 110.9 | 23.81 | 0.76 | 0.4 | 42 | 41 | 240 | 0.81 | 296 | 50 | 0.16 | 0.14 | 4.8 | 0.9 | 9.6 | 0.0012 |
| | | 2 | 4-6/12 | 111.0 | 20.96 | 0.76 | 1.7 | 63 | 66 | 268 | 0.84 | 319 | 39 | 0.24 | 0.19 | 6.8 | 1.6 | 5.3 | 0.0121 |
| | | 3 | 4-8/12 | 112.0 | 23.25 | 0.76 | 3.1 | 35 | 49 | 296 | 0.87 | 340 | 30 | 0.12 | 0.10 | 9.9 | 1.1 | 6.0 | 0.0134 |
| | | 4‖ | 4-9/12 | 112.0 | 21.27 | 0.76 | 4.0 | 52 | 66 | 264 | 0.84 | 315 | 30 | 0.20 | 0.17 | 8.9 | 1.6 | 7.4 | 0.0085 |
| | | 5 | 4-11/12 | 113.1 | 22.93 | 0.83 | 5.5 | 54 | 59 | 174 | 0.73 | 238 | 37 | 0.31 | 0.22 | 4.7 | 1.2 | 5.4 | 0.0128 |
| 2 | C. P. ♀ | 1 | 1-4/12 | 81.0 | 10.24 | 0.44 | 0.6 | 10 | 22 | 125 | 0.63 | 199 | 14 | 0.09 | 0.09 | 9.2 | 1.1 | 3.8 | 0.0113 |
| | | 2 | 1-8/12 | 81.0 | 10.32 | 0.44 | 4.5 | 19 | 21 | 85 | 0.52 | 163 | 8 | 0.23 | 0.12 | 10.2 | 2.6 | 3.5 | 0.0063 |
| | | 3‖ | 1-8/12 | 83.0 | 8.86 | 0.43 | 5.2 | 43 | 49 | 215 | 0.78 | 276 | 22 | 0.21 | 0.16 | 9.7 | 1.9 | 2.4 | 0.0141 |
| 3 | A. G. ♀ | 1 | 1-3/12 | 80.0 | 14.28 | 0.50 | 1.0 | | 19 | 581 | 0.90 | 646 | 18 | 0.03 | 0.03 | 32.7 | 1.1 | 3.8 | 0.0171 |
| | | 2 | 2-4/12 | 81.2 | 11.23 | 0.49 | 14.0 | 10 | 11 | 12¶ | 0.13 | 106 | 1** | 0.80 | 0.10 | 12.9 | 6.5 | 2.4 | 0.0120 |
| 4 | L. A. ♀ | 1 | 2-3/12 | 89.6 | 12.11 | 0.52 | 1.0 | 48 | 62 | 208 | 0.77 | 270 | 43 | 0.23 | 0.18 | 4.9 | 1.0 | 3.8 | 0.0136 |
| 5 | R. S. ♂ | 1 | 6-6/12 | 123.0 | 33.90 | 0.98 | 1.0 | | 44 | 545 | 0.90 | 605 | 45 | 0.08 | 0.07 | 12.0 | 1.0 | 11.1 | 0.0134 |
| | | 2 | 7-8/12 | 125.1 | 29.19 | 0.95 | 14.0 | 9 | 8 | 20 | 0.20 | 96 | 1 | 0.44 | 0.09 | 27.9 | 14.3 | 10.1 | 0.0039 |
| 6 | R. M. ♂ | 1 | 2-7/12 | 91.5 | 17.75 | 0.61 | 1.5 | | 37 | 399 | 0.89 | 448 | 31 | 0.09 | 0.08 | 12.7 | 1.2 | 5.0 | 0.0084 |
| 7 | B. D. ♀ | 1 | 1-8/12 | 87.0 | 14.75 | 0.50 | 2.0 | | 48 | 303 | 0.87 | 349 | 31 | 0.16 | 0.14 | 9.7 | 1.5 | 6.5 | 0.0073 |
| 8 | G. B. ♂ | 1 | 1-10/12 | 90.8 | 17.70 | 0.54 | 2.0 | 33 | 51 | | | | | | | | | 8.3 | |
| | | 2‖ | 1-11/12 | 91.5 | 11.88 | 0.54 | 2.8 | 78 | 93 | 325 | 0.86 | 378 | 55 | 0.21 | 0.21 | 5.9 | 1.1 | 3.2 | 0.0191 |
| | | 3 | 2-5/12 | 94.6 | 21.47 | 0.65 | 8.8 | 15 | 20 | 168 | 0.71 | 237 | 17 | 0.09 | 0.06 | 9.7 | 0.9 | 7.3 | 0.0101 |
| 9 | H. M. ♂ | 1 | 3-4/12 | 105.4 | 17.86 | 0.71 | 2.5 | 57 | 69 | 270 | 0.85 | 318 | 52 | 0.21 | 0.20 | 5.2 | 1.0 | 5.5 | 0.0143 |
| | | 2 | 3-8/12 | 106.1 | 24.63 | 0.73 | 5.0 | 55 | 65 | 198 | 0.76 | 260 | 38 | 0.28 | 0.21 | 5.2 | 1.2 | 8.2 | 0.0143 |
| 10 | J. M. ♀ | 1 | 4-8/12 | 110.6 | 23.41 | 0.70 | 4.0 | | 95 | 471 | 0.90 | 524 | 44 | 0.20 | 0.18 | 10.8 | 2.2 | 12.0 | 0.0108 |
| | | 2 | 5-11/12 | 111.2 | 23.36 | 0.81 | 18.0 | 14 | 13 | 34 | 0.33 | 103 | 1 | 0.40 | 0.13 | 34.0 | 14.0 | 6.9 | 0.0087 |

TABLE III—*Continued*

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Patient data | | | | | | | | Renal functions/M² (average) | | | | | | | | | | V$_D$ Thio.‡ | |
| Case number | Patient | Determination number | Age (*years*) | Height (*cm.*) | Weight (*Kg.*) | Surface area (*M²*) | Duration of disease (*months*) | GFR inulin *ml./min.* | GFR $Na_2S_2O_3$ *ml./min.* | RPF *ml./min.* | Est. extract. ratio† | RPF$_e$† *ml./min.* | TmPAH | $\frac{GFR}{RPF}$ | $\frac{GFR}{RPF_e}$† | $\frac{RPF}{TmPAH}$ | $\frac{GFR}{TmPAH}$ | Liters | λe |
| Normal values: | | 25 Children (49)§<br>1-5/12–10-1/12<br>61 Adults (22) | | SA =0.48–1.24<br>SA =1.48–1.91 | | 1.00<br>1.00 | | 76±15<br>75±12 | 74(19) | 364±69<br>403±78 | 0.89<br>0.90 | 410±70<br>448 | 42±10<br>47 | 0.21±.05<br>0.19±.02 | 0.19<br>0.17 | 9.0±3.5<br>8.6 | 2.1±0.9<br>1.7 | | |
| 11 | F. K. ♂ | 1 | 2-3/12 | 93.4 | 16.22 | 0.46 | 4.0 | | 121 | 220 | 0.78 | 282 | 11 | 0.55 | 0.43 | 19.2 | 10.6 | 9.8 | 0.0133 |
| | | 2 | 3-2/12 | 95.0 | 14.77 | 0.50 | 16.0 | 2 | 3 | 10 | 0.11 | 93 | 1 | 0.23 | 0.03 | 19.6 | 4.1 | 8.8 | 0.0000 |
| 12 | J. Ma. ♂ | 1 | 3-0/12 | 100.2 | 23.74 | 0.65 | 4.0 | 20 | 23 | 240 | 0.81 | 297 | 32 | 0.08 | 0.07 | 7.5 | 0.7 | 7.2 | 0.0097 |
| | | 2‖ | 3-1/12 | 100.6 | 15.62 | 0.65 | 4.7 | 68 | 90 | 279 | 0.85 | 328 | 37 | 0.24 | 0.21 | 7.5 | 1.5 | 4.4 | 0.0165 |
| 13 | J. F. ♀ | 1 | 3-11/12 | 96.1 | 16.06 | 0.62 | 4.0 | | 32 | 287 | 0.86 | 334 | 22 | 0.11 | 0.10 | 13.1 | 1.5 | 5.3 | 0.0104 |
| | | 2 | 5-0/12 | 98.0 | 14.49 | 0.60 | 17.0 | 13 | 15 | 114 | 0.60 | 190 | 6 | 0.12 | 0.07 | 18.4 | 2.2 | 4.3 | 0.0035 |
| 14 | G. C. ♂ | 1 | 2-1/12 | 92.0 | 15.00 | 0.56 | 5.0 | 49 | 40 | 272 | 0.85 | 320 | 23 | 0.14 | 0.13 | 12.0 | 1.7 | 5.5 | 0.0059 |
| 15 | R. G. ♂ | 1 | 3-1/12 | 90.1 | 15.81 | 0.54 | 6.0 | | 23 | 178 | 0.73 | 243 | 12 | 0.13 | 0.09 | 14.6 | 1.9 | 5.6 | 0.0103 |
| | | 2 | 3-3/12 | 91.0 | 14.61 | 0.56 | 7.5 | | 25 | 258 | 0.83 | 310 | 30 | 0.10 | 0.08 | 8.6 | 0.8 | 4.8 | 0.0113 |
| 16 | P. N. ♀ | 1 | 2-8/12 | 91.5 | 15.07 | 0.52 | 6.0 | | 103 | 442 | 0.90 | 490 | 38 | 0.23 | 0.21 | 11.7 | 2.8 | 6.0 | 0.0217 |
| 17 | R. B. ♂ | 1‖ | 2-1/12 | 85.5 | 13.33 | 0.52 | 7.0 | 92 | 96 | 239 | 0.78 | 307 | 20 | 0.43 | 0.33 | 12.3 | 6.2 | | |
| | | 2 | 2-5/12 | 88.0 | 18.71 | 0.55 | 11.0 | 19 | 20 | 136 | 0.65 | 209 | 9 | 0.14 | 0.09 | 14.5 | 2.1 | 6.2 | 0.0076 |
| | | 3‖ | 2-6/12 | 89.3 | 13.37 | 0.55 | 12.0 | 51 | 47 | 127 | 0.63 | 202 | 9 | 0.30 | 0.25 | 14.5 | 4.6 | 3.7 | 0.0152 |
| 18 | E. D. ♀ | 1 | 2-6/12 | 91.0 | 20.20 | 0.56 | 7.0 | | 2 | 88 | 0.53 | 167 | 21 | 0.03 | 0.02 | 4.2 | 0.1 | | |
| | | 2†† | 2-7/12 | 91.0 | 13.91 | 0.58 | 8.0 | | 4 | 111 | 0.59 | 187 | 23 | 0.04 | 0.02 | 4.7 | 0.2 | | |
| | | 3 | 3-10/12 | 92.9 | 17.55 | 0.60 | 22.0 | 4 | 4 | 12 | 0.14 | 88 | 1 | 0.35 | 0.05 | 14.4 | 4.6 | 3.7 | 0.0078 |
| 19 | H. S. ♂ | 1 | 3-0/12 | 100.4 | 21.58 | 0.71 | 8.0 | 24 | 27 | 115 | 0.60 | 191 | 7 | 0.21 | 0.13 | 15.8 | 2.9 | 6.2 | 0.0105 |
| | | 2 | 4-3/12 | 101.9 | 20.13 | 0.71 | 12.7 | 31 | 36 | 129 | 0.64 | 202 | 6 | 0.23 | 0.15 | 22.5 | 5.0 | 5.7 | 0.0132 |
| 20 | D. G. ♀ | 1 | 7-8/12 | 127.5 | 40.90 | 1.00 | 9.0 | | 18 | 119 | 0.61 | 196 | 9 | 0.15 | 0.09 | 13.4 | 2.0 | 4.4 | 0.0206 |
| 21 | L. C. ♀ | 1 | 3-11/12 | 99.0 | 15.86 | 0.64 | 15.0 | 59 | 99 | 308 | 0.87 | 353 | 49 | 0.19 | 0.17 | 6.3 | 1.3 | 3.3 | 0.0226 |

TABLE III—*Continued*

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Patient data | | | | | | | | Renal functions/$M^2$ (average) | | | | | | | | | | $V_D$ Thio.‡ | |
| Case number | Patient | Determination number | Age (*years*) | Height (*cm.*) | Weight (*Kg.*) | Surface area ($M^2$) | Duration of disease (*months*) | GFR inulin *ml./min.* | GFR $Na_2S_2O_3$ *ml./min.* | RPF *ml./min.* | Est. extract. ratio† | $RPF_e$† *ml./min.* | TmPAH | $\frac{GFR}{RPF}$ | $\frac{GFR}{RPF_e}$† | $\frac{RPF}{TmPAH}$ | $\frac{GFR}{TmPAH}$ | Liters | λc |
| Normal values: | | 25 Children (49)§<br>1-5/12–10-1/12<br>61 Adults (22) | | SA =0.48–1.24<br>SA =1.48–1.91 | | 1.00<br>1.00 | | 76±15<br>75±12 | 74(19) | 364±69<br>403±78 | 0.89<br>0.90 | 410±70<br>448 | 42±10<br>47 | 0.21±.05<br>0.19±.02 | 0.19<br>0.17 | 9.0±3.5<br>8.6 | 2.1±0.9<br>1.7 | | |
| 22 | H. O.<br>♀ | 1<br>2 | 14-6/12<br>15-6/12 | 159.3<br>160.8 | 60.40<br>56.91 | 1.63<br>1.55 | 15.0<br>26.0 | <br>19 | 39<br>23 | 349<br>178 | 0.88<br>0.73 | 397<br>244 | 48<br>17 | 0.11<br>0.11 | 0.10<br>0.09 | 7.3<br>10.8 | 0.8<br>1.2 | 12.8<br>17.2 | 0.0198<br>0.0098 |
| 23 | W. L.<br>♂ | 1 | 4-1/12 | 93.0 | 13.96 | 0.54 | 15.5 | | 8 | 12 | 0.14 | 88 | 2** | 0.62 | 0.09 | 5.1 | 3.1 | 6.2 | 0.0047 |
| 24 | E. G.<br>♀ | 1<br>2 | 4-2/12<br>5-3/12 | 101.0<br>108.9 | 23.04<br>17.73 | 0.76<br>0.73 | 17.0<br>30.0 | <br>47 | 12<br>50 | 183<br>175 | 0.65<br>0.73 | 281<br>239 | 8<br>31 | 0.06<br>0.27 | 0.04<br>0.20 | 23.3<br>5.6 | 1.5<br>1.4 | 5.3<br>4.6 | 0.0159<br>0.0143 |
| 25 | S. S.<br>♀ | 1<br>2‡‡ | 4-11/12<br>4-11/12 | 104.0<br>104.0 | 18.74<br>19.96 | 0.70<br>0.70 | 18.5<br>19.0 | 26<br>26 | 28 | 182<br>134 | 0.74<br>0.65 | 246<br>207 | 28<br>14 | 0.14<br>0.20 | 0.11<br>0.13 | 6.5<br>9.8 | 0.8<br>2.1 | 5.5 | 0.0098 |
| 26 | B. S.<br>♀ | 1 | 5-1/12 | 101.0 | 22.05 | 0.71 | 20.0 | | 10 | 22 | 0.21 | 104 | 3 | 0.48 | 0.10 | 6.7 | 3.2 | 6.1 | 0.0048 |
| 27 | L. N.<br>♀ | 1 | 4-2/12 | 93.0 | 13.85 | 0.59 | 22.0 | | 10 | 18 | 0.20 | 90 | 4 | 0.53 | 0.11 | 4.0 | 2.1 | 3.4 | 0.0104 |
| 28 | E. M.<br>♀ | 1 | 4-6/12 | 94.0 | 20.86 | 0.62 | 35.0 | | 4 | 19 | 0.20 | 91 | 3 | 0.19 | 0.04 | 6.7 | 1.3 | 7.6 | 0.0087 |
| 29 | A. O.<br>♀ | 1 | 7-10/12 | 115.2 | 22.63 | 0.84 | 64.0 | 49 | 58 | 255 | 0.84 | 304 | 53 | 0.19 | 0.17 | 4.8 | 0.9 | 5.6 | 0.0131 |

* The order of patients and observations coincides with that of Table II—*e.g.*, according to duration of disease at time of initial study.

† Data in these columns based upon estimated extraction ratios; *cf.* text for limitations of such inference.

‡ Uncorrected for surface area.

§ Renal function studies on four "normal" children by our techniques gave similar values.

‖ Measurements obtained following ACTH induced diuresis.

¶ Despite infusion of minimal dose of PAH, serum levels of 10–11 mg. % were obtained. Estimate of PAH clearance may be invalid.

** One negative Tm period not included.

†† Values obtained when edema-free following paracentesis.

‡‡ Measurements obtained following ACTH, but before diuresis.

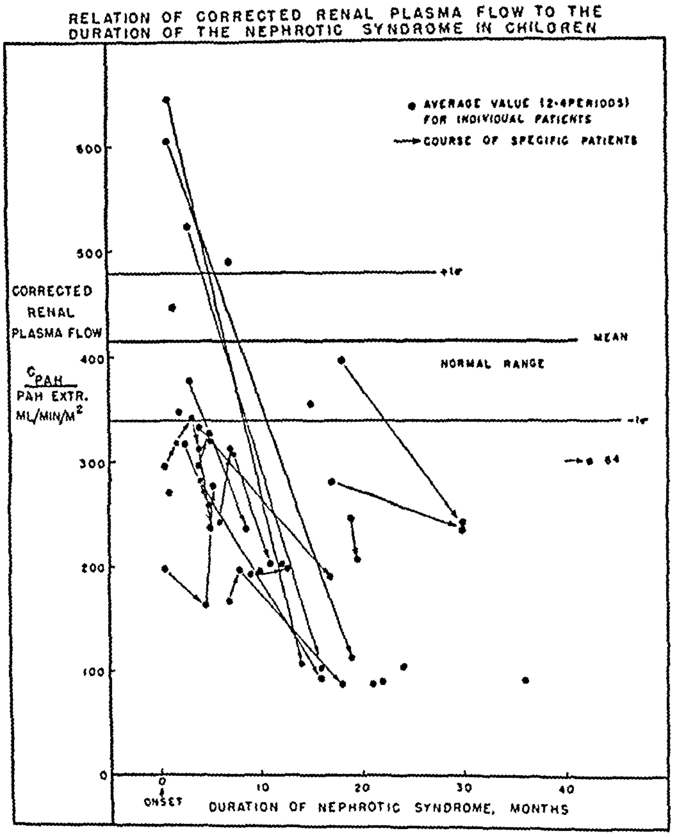

FIG. 6.

general, glomerular function in the nephrotic syndrome varied with duration of the disease. Uniformly reduced filtration rates were noted in nine patients observed during the initial 2.5 months of the disease. Occasionally, supernormal values were noted in the following few months. More commonly, progressive reduction occurred.

Diuresis, which occurs spontaneously in many nephrotic children during the first weeks or months of the disease, usually was associated with improvement of GFR. Increased GFR and filtration fraction were not essential to initiate diuresis (42). In one instance (No. $1_2$),[10] the initial diuresis was spontaneous; in others (Nos. $8_2$, $12_2$, $17_{1,3}$), it followed ACTH therapy. Following the initial diuresis and during the phase of recurring edema (Nos. $1_3$, $8_3$) reduced filtration was again noted. Four instances of supernormal filtration rates were observed after the first two months of the nephrotic syndrome. In one patient (No. $17_1$), the observation was preceded by diuresis initiated with ACTH therapy. In others, (Nos. 10, 11, 16), no therapy had been employed. Subsequent determinations (Nos. $17_2$, $10_2$, $11_2$) revealed striking reduction of filtration. An increase in filtration rate was observed twice in the same patient (Nos. $1_4$, $17_3$) following successive courses of treatment with ACTH with diuresis. Spontaneous or ACTH induced improvement of GFR after many months of active disease was also noted twice (Nos. $19_2$, $24_2$).

With continuation of the disease, progressive lowering of GFR was ultimately associated with renal failure. Extreme reduction in GFR was noted (No. $18_{1,2}$) for many months before clinical evidence of renal failure became apparent. Demonstration of diminished filtration after 17 months of active disease did not preclude apparent clinical remission, (No. 24). Subsidence of nephrotic symptoms and signs after a protracted course was usually characterized by persistent evidence of minimal inflammatory renal response and slightly reduced GFR (Nos. $17_3$, $24_2$, 29). Relatively early clinical remission was accompanied by normal GFR (No. 21).

### *Renal plasma flow*

Unlike GFR, greater than normal values for apparent renal plasma flow were occasionally observed during the first months of the disease (Figure 6). Marked elevation of renal plasma flow was observed concomitantly with supernormal GFR (Nos. 10, 16) as well as with moderately (No. $5_1$), or markedly (No. 3) reduced GFR. In several instances, elevation of the filtration rate was associated with diminution of renal plasma flow (Nos. $11_1$, $17_1$). It was evident that change in renal plasma flow could occur without significant variation in GFR (Nos. $1_5$, 3, 9, 15, 18, 22). The reverse also was true (Nos. 1, 12, 17).

Following diuresis, either spontaneous (No. $1_2$) or induced (Nos. $1_4$, $17_3$, $12_2$), a definite increase in renal plasma flow usually was not observed. In one instance (No. $2_3$), RPF was apparently doubled. While normal values were occasionally noted with apparent clinical remission (Nos. 12, 21), reduced renal plasma flow was also observed (Nos. $24_2$, 29). Persistence of the nephrotic syndrome was usually associated with progressive reduction in both measured and "corrected" renal plasma flow (Nos. 5, $8_3$, 13, $17_2$, 18, 22).

### *Maximal tubular excretory capacity*

Maximal tubular excretory capacity was usually within normal limits or was slightly reduced during

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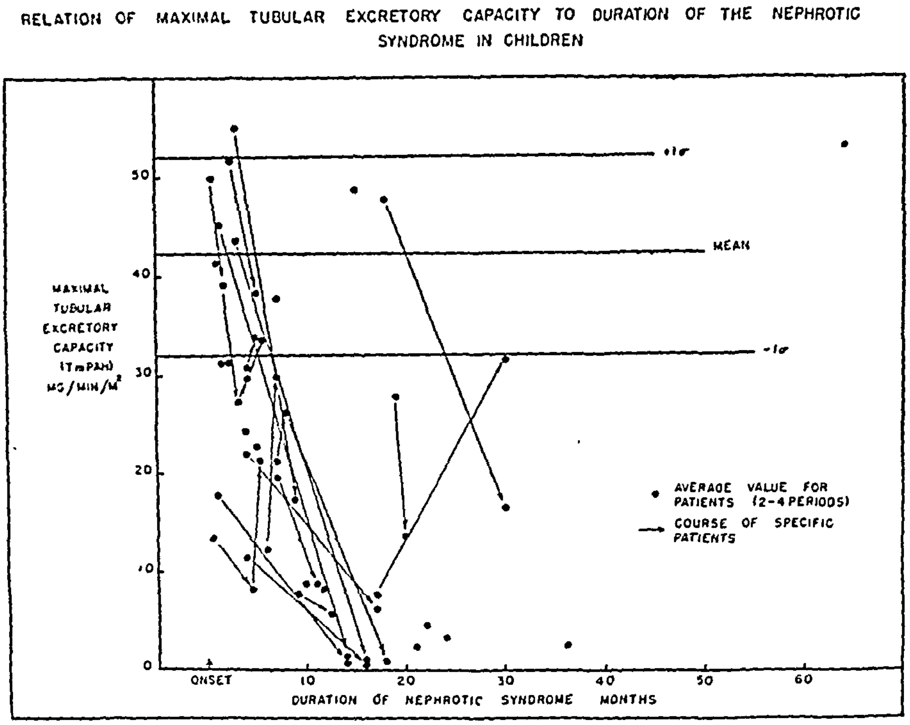
[10] Subscript refers to determination number of a given patient; in this instance, to the second renal function study of patient S. J.



RELATION OF MAXIMAL TUBULAR EXCRETORY CAPACITY TO DURATION OF THE NEPHROTIC SYNDROME IN CHILDREN

Fig. 7.

the first months of the disease (Figure 7). Neither spontaneous nor ACTH induced diuresis commonly was associated with significant alteration in this function (Nos. $1_{2,4}$, $12_2$, $17_3$) except in (No. $2_3$). Early reduction in maximal tubular excretory capacity was apparently reversible, (Nos. $2_3$, $15_2$). With persistence of the disease, progressive reduction in Tm$_{PAH}$ was observed. With clinical remission, despite a protracted course, normal values were observed (Nos. 21, 24, 29). Tubular reabsorption of PAH possibly accounts for the occasional observation of negative Tm periods (Nos. 3, 23) or apparent extreme reduction of effective tubular mass (Nos. 5, 10, 11, 18, 26–28).[11]

## DISCUSSION

Marked distortion of the nephron and its supporting structures with extreme variation in architecture is not common in the early phases of the nephrotic syndrome in children (43, 44). In instances where death has resulted from intercurrent infection in the early months of the disease, the absence of striking morphologic changes, particularly of the glomerulus, has been evident (9, 11). Our data indicate a slight to moderate reduction of glomerular filtration early in the course of the nephrotic syndrome. Filtration is largely the resultant of hydrostatic pressure in one direction, oncotic and renal interstitial pressure in the other, and of the area over which the resultant pressure is exerted. Changes in effective renal arteriolar resistance may modify filtration (45, 46). The ratio of glomerular filtration to renal plasma flow (filtration fraction) defines the filtration equilibrium. Early in the nephrotic syndrome, the filtration fraction (FF) occasionally was observed to be essentially normal; rarely, it was markedly elevated; most commonly it was reduced (Figure 8). Renal plasma flow was usually reduced early in the disease, but filtration appeared to suffer greater proportionate reduction, although inconstant dissociation of the two functions was evident (Figure 9). The relationship between the two variables is illustrated by means of a correlation diagram of the sort employed by Goldring and his associates (47) and Smith (48). In this diagram, the elipse represents the perimeter of a normal surface within which 70% of normally correlated values would be expected to fall. The statistical background for the elipse was calculated from the data of Rubin, Bruck, and Rapoport (49). Spontaneous or induced (ACTH) improvement of the nephrotic syndrome was usually associated with an increased filtration fraction. An absolute increase of GFR usually was responsible. Plasma protein concentration (or content) did not appear to undergo appreciable diminution with diuresis, and possibly increased. Therefore, further reduction of oncotic pressure accounting for increased filtration seemed unlikely. An increased filtration fraction associated with reduced afferent and augmented efferent arteriolar resistance has been observed with diuresis in nephrotic edema (42). Improved filtration during the nephrotic syndrome might result from decreased interstitial renal (subcapsular) pressure, alteration in membrane permeability, increase of the effective glomerular bed by restoration of normal circulatory channels, or by increase in glomerular pressure. It is not possible to choose between these alternatives at present. Since reduced GFRs were demonstrably reversible for variable periods of time, a disease process irreversibly obliterating glomeruli during the relatively early phases of the nephrotic syndrome seems unlikely. Persistence of the nephrotic syndrome with evidence of active renal disease for some period of time usually was associated with gradual reduction in all functional moities. When, in cases of the protracted nephrotic syndrome progressive diminution of filtration fraction was observed, associated changes in $\text{RPF}/\text{Tm}_{\text{PAH}}$ and $\text{GFR}/\text{Tm}_{\text{PAH}}$ suggested extensive nephron damage. These observations are consistent with those reported in glomerulonephritis (14, 15).

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[11] Despite rising serum PAH and inulin levels, consecutive periods commonly revealed little change in Tm although a slight tendency toward depression of simultaneous inulin clearance was noted.

Increased maximal tubular excretory capacity following diuresis was occasionally observed in the first few months of the nephrotic syndrome. These observations suggest increased activity of the tubular cell. It has been demonstrated that the excretory capacities of the tubular cell may be altered under certain conditions (50). In either case, the concept of primary degenerative, irreversible change of the tubular tissue seems untenable. With clinical remission following many months of an active, persistent nephrotic syndrome, the effective tubular mass appeared to be essentially normal or only slightly reduced. With relapse, or progressive, active disease, reduction of Tm appeared to correlate with relatively symmetrical reduction of GFR (Figure 10). This phenomenon has been interpreted as evidence for ablation of functioning nephrons (48). The simultaneous appearance of biochemical renal insufficiency (Table II) tended to corroborate this interpretation. Reduction of

RELATION OF FILTRATION FRACTION TO DURATION OF DISEASE IN CHILDREN WITH THE NEPHROTIC SYNDROME

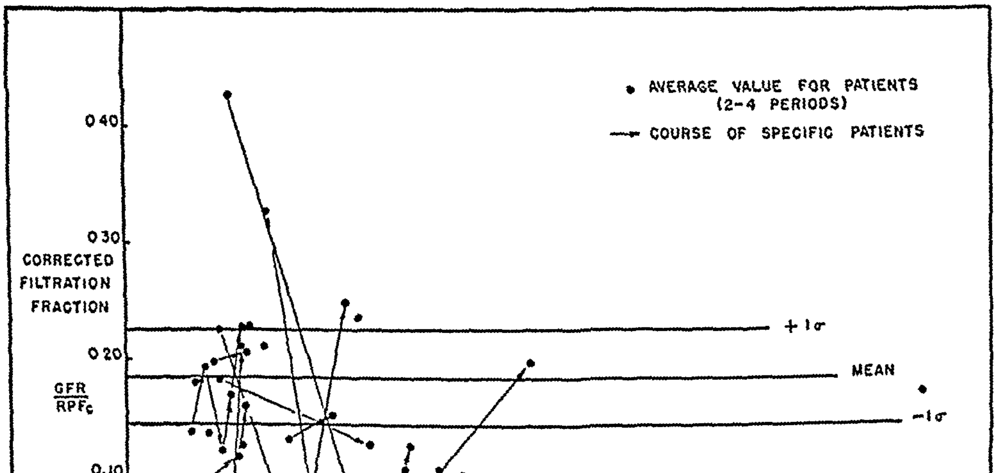

FIG. 8.

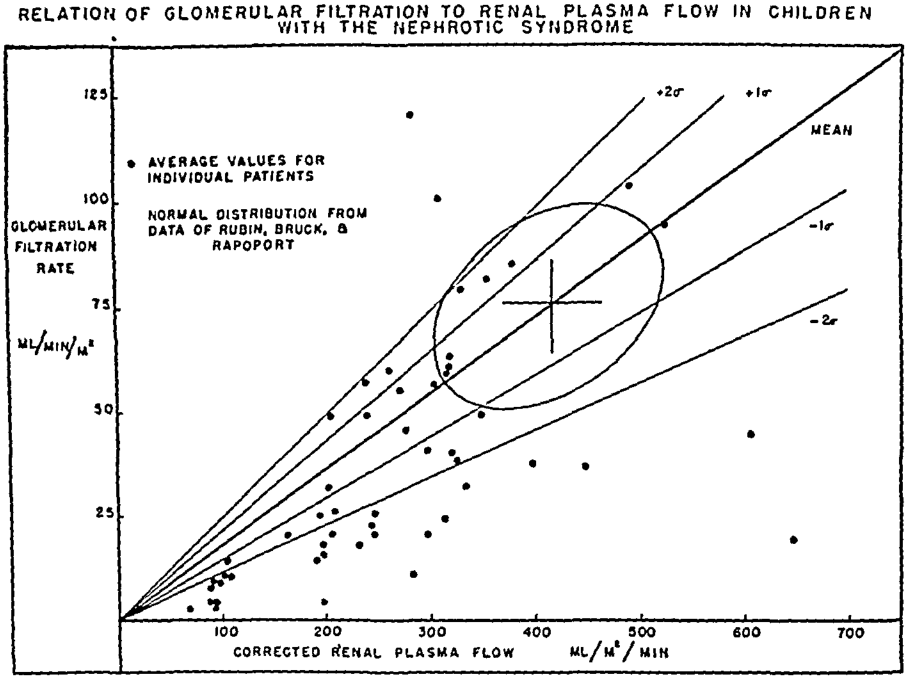
RELATION OF GLOMERULAR FILTRATION TO RENAL PLASMA FLOW IN CHILDREN WITH THE NEPHROTIC SYNDROME

FIG. 9.

effective tubular mass, however, appeared disproportionately greater than diminution of renal plasma flow (Figure 11), suggesting hyperemia of the residual tubular tissue. In view of pathologic studies indicating distortion, vascular obliteration (51), and relative renal ischemia in advanced renal disease (15, 52), it seems likely that the apparent hyperemia is vicarious.

Neither the clinical observations nor the renal functional data clarify the etiology or pathogenesis of salient features of the nephrotic syndrome such as edema, hyperlipemia, hypoproteinemia or hypocalcemia. However, the data of this report form the basis for an hypothesis concerning the renal hemodynamic adjustments in the nephrotic syndrome in children (Figure 12). The validity of clearance measurements in the presence of nephron disease and at very reduced levels of renal function is uncertain and interpretation hazardous. With this reservation, further qualified by limitations of sampling and the necessity for protracted observation, it is suggested that the initial renal insult, which may be of immunogenic nature (53), involves the entire nephron in variable degree, with early alteration of its functional capacities. Preponderance of glomerular or tubular dysfunction may be observed. Initial reduction of GFR and FF may be contingent upon increased renal interstitial pressure, impaired glomerular membrane permeability, cortico-medullary shunts bypassing some glomeruli, or reduction of glomerular pressure. Diminished oncotic pressure would tend to improve glomerular filtration. Physiologic adjustment might attend reduction of subcapsular and increased intra-glomerular pressures. Constriction of the efferent arterioles, if associated with some dilatation of the afferent arterioles, reduced renal interstitial pressure and minimal alteration of the glomerular membrane, could theoretically induce supernormal glomerular filtration. Successful adjustment of the metabolic activities of tubular cells might result in diuresis and at least temporary remission. Recovery would follow successful, persistent functional reorganization of the nephron before irreversible anatomic damage was established. Failing functional reorganization, or possibly with recurrent stimulation of sensitized renal tissues by a primary etiologic agent, gradual distortion of the vascular architecture and dependent nephron structures characteristic of chronic renal disease might then initiate a vicious cycle of glomerular obliteration with diminished tubule blood flow and vascular shunting. Relatively increased blood flow adjacent to residual tubular tissue would thus represent

RELATION OF GLOMERULAR FILTRATION RATE TO EFFECTIVE TUBULAR MASS IN CHILDREN WITH THE NEPHROTIC SYNDROME

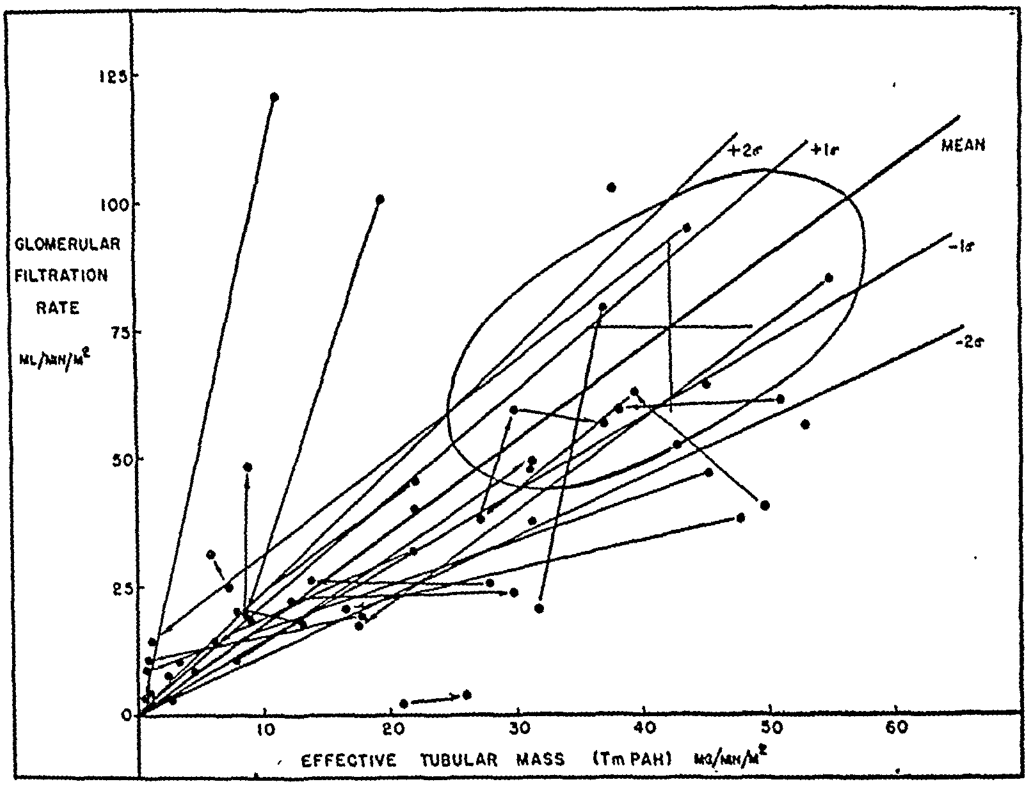

Fig. 10.

RELATION OF RENAL PLASMA FLOW TO EFFECTIVE TUBULAR MASS IN CHILDREN WITH THE NEPHROTIC SYNDROME

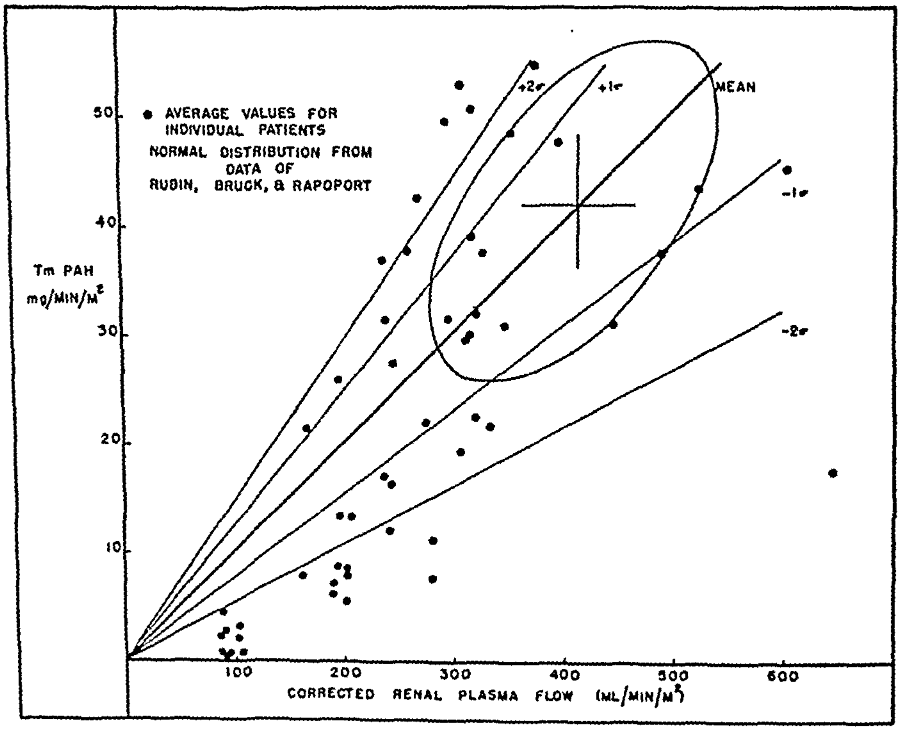

Fig. 11.

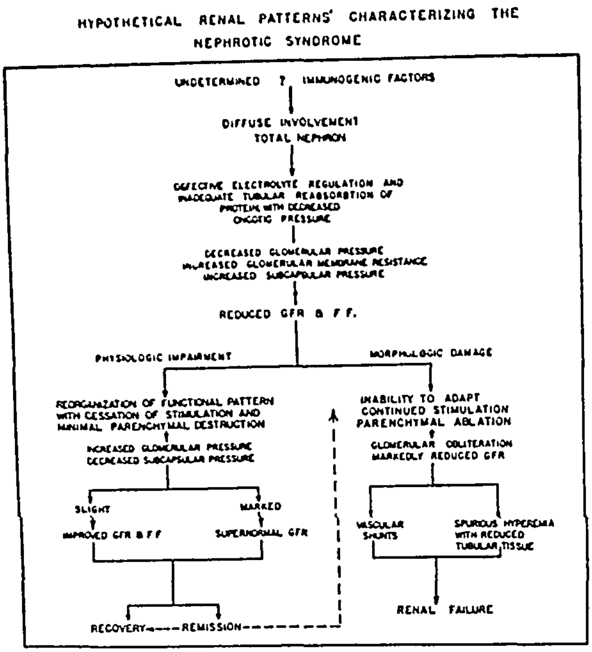
HYPOTHETICAL RENAL PATTERNS CHARACTERIZING THE NEPHROTIC SYNDROME
UNDETERMINED ? IMMUNOGENIC FACTORS
DIFFUSE INVOLVEMENT TOTAL NEPHRON
DEFECTIVE ELECTROLYTE REGULATION AND INADEQUATE TUBULAR REABSORBTION OF PROTEIN WITH DECREASED ONCOTIC PRESSURE
DECREASED GLOMERULAR PRESSURE INCREASED GLOMERULAR MEMBRANE RESISTANCE INCREASED SUBCAPSULAR PRESSURE
REDUCED GFR & F F.
PHYSIOLOGIC IMPAIRMENT
MORPHOLOGIC DAMAGE
REORGANIZATION OF FUNCTIONAL PATTERN WITH CESSATION OF STIMULATION AND MINIMAL PARENCHYMAL DESTRUCTION
INCREASED GLOMERULAR PRESSURE DECREASED SUBCAPSULAR PRESSURE
SLIGHT
MARKED
IMPROVED GFR & F F
SUPERNORMAL GFR
RECOVERY
REMISSION
INABILITY TO ADAPT CONTINUED STIMULATION PARENCHYMAL ABLATION
GLOMERULAR OBLITERATION MARKEDLY REDUCED GFR
VASCULAR SHUNTS
SPURIOUS HYPEREMIA WITH REDUCED TUBULAR TISSUE
RENAL FAILURE

FIG. 12.

a spurious hyperemia and an indication of impending renal failure.

Since young children, unlike older patients, often recover following the nephrotic syndrome, the more favorable tissue reactivity of the young organism may explain the different course of this disease in these two age groups.

## SUMMARY AND CONCLUSIONS

Twenty-nine children with the nephrotic syndrome, a fairly typical sample of 90 patients recently observed, have been studied by clinical, biochemical, and renal function techniques.

Conventional clinical criteria frequently proved unreliable early in the course of the nephrotic syndrome as indices of the functional status of the kidney or as guides to the subsequent course of the patient. The triad of anemia, acidosis, and azotemia, when correlated with a diminished GFR, indicated incompetence of the nephron for biochemical regulation and probable morphologic damage with poor prognosis.

Fluctuating changes in GFR, RPF, and $Tm_{PAH}$ during the nephrotic syndrome suggested that variations in these measurements are a function of the severity and duration, rather than type, of disease. The biochemical and renal functional patterns indicate that reversible metabolic and hemodynamic rather than irreversible anatomic nephron damage frequently occurs. With progressive disease of long duration, markedly impaired renal function suggested that irreversible parenchymal destruction gradually supersedes the earlier physiologic changes, with culmination in renal failure.

The primary etiologic mechanism remains undetermined; however, the idiopathic nephrotic syndrome in children, whether called "pure or lipoid nephrosis," "mixed nephritis," or "nephrotic stage of subacute or chronic glomerulonephritis," appears to be a form of nephritis in which functional alteration and eventual anatomical changes indicate that the glomerulus is a primary site of the disease process. With inhibition of the unknown etiologic mechanism presumably before extensive anatomic damage has supervened, spontaneous or induced remissions may proceed to apparent recovery.

## ACKNOWLEDGMENTS

We are deeply indebted to the house staff, the nursing staff and the clinical laboratories of the Children's Medical Center for their cooperation and assistance in the care of these children. We also wish to acknowledge the valuable assistance rendered by Lewis A. Barness and Malcolm Holliday in some of the initial renal function procedures. The manuscript profited from the constructive criticism of Drs. Herbert Chasis and Homer W. Smith. We sincerely appreciate their comments.

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# STUDIES IN DISORDERS OF MUSCLE. VII. CLINICAL MANIFESTATIONS AND INHERITANCE OF A TYPE OF PERIODIC PARALYSIS WITHOUT HYPOPOTASSEMIA[1]

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(Submitted for publication December 13, 1950; accepted, March 12, 1951)

## INTRODUCTION AND REVIEW OF LITERATURE

The report by several investigators in 1937 and 1938 that the paralytic attacks in the disease, periodic paralysis, were associated with depressed serum potassium values led to the conclusion that the disorder is the consequence of some fault in the regulation of serum potassium levels (1–4). Ample support for such a concept appeared to be given by other observations. Thus, depletion of serum potassium in various other clinical circumstances, as in the recovery from diabetic acidosis and in the Addisonian patient overtreated with desoxycorticosterone acetate, is associated with flaccid paralysis. Again, it is known that hypopotassemia results in cardiac arrest in diastole and causes impaired contractility of muscle *in vitro*. Finally, the balance studies of Danowski and associates (5) showed that, in the patients they studied, the attacks of paralysis were preceded by the movement of potassium into the intracellular phase and that urinary potassium excretion as well as the serum potassium concentration decreased.

Talbot (6) in 1941 summarized the data concerning more than 400 cases of periodic paralysis. The disorder has been usually referred to as familial or family periodic paralysis. It seems best to us as it did to Talbot to call the disease periodic paralysis, specifying the hereditary nature of the disorder when it is genetically determined, and referring to the other cases as sporadic, associated with thyrotoxicosis or in other ways specifying the mechanism involved where it is known. Talbot concluded that the syndrome probably represented a clinical entity due to a single metabolic dysfunction.

In our study of a kindred in which 33 individuals with clinically typical periodic paralysis were known to have occurred, we observed no striking hypopotassemia during attacks in the patients whom we studied in detail. This led us to undertake a critical review of the data dealing with this disorder in the literature. A poor correlation between the onset and the severity of the attacks and the magnitude of the depression of serum potassium values has been noted and commented upon by several authors, including Talbot (6). Occasional instances of paralysis without depression of serum potassium levels have been reported (7). Furthermore, the levels of serum potassium found during attacks in patients with periodic paralysis usually have not been as low as those observed in patients who do not carry this genetic trait but who have hypopotassemic paralysis resulting from better understood metabolic abnormalities (3, 6, 8, 9). A corollary observation is the apparent lack of specificity of potassium in relieving or preventing the paralysis in certain patients with periodic paralysis in contrast to its excellent effect in the other metabolic hypokaliemias (1, 6, 10, 11).

It seems probable, therefore, that periodic paralysis as described in the literature is produced by more than one mechanism or that the serum potassium depletion is not the fundamental disorder. Such anomalies as abnormal storage of glycogen as well as other changes in intracellular potassium metabolism might be responsible for these vagaries of the serum potassium levels. The lack of complete similarity of the hereditary cases suggests that different genetic mechanisms are involved, most probably multiple mutations. These mutations can be understood best by studying them in kindreds where the expression of the same gene in many individuals may be observed. This disorder, like many other human hereditary disorders

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[1] This study was supported in part by a grant from the United States Public Health Service.

such as hereditary muscular dystrophy, hereditary blindness, hereditary deafness, etc., must be broken down into specific genetic entities before it can be analyzed and interpreted adequately.

An examination of the genetic literature illustrates the diversity and variety of expression of periodic paralysis. The trait has been reported to be a dominant with skipped generations (12, 13), a complete dominant (14, 15), a recessive (6), and a sex-linked recessive (16). The gene has been reported as manifesting incomplete penetrance in females (6) and as having complete penetrance in both sexes (15). The expressivity may range from a slight recurrent weakness in one limb, as reported in one family (10), to paralysis so severe that nearly the entire body is paralyzed, in other families (13). Again, the paralysis may be so extreme in some cases as to cause death (13, 14).

The duration of an attack in the members of some families may range from a few minutes to several days while in other families an individual may never be affected for more than a few hours (6). In certain families the attacks are rare, occurring once or twice in a lifetime or two or three times in a year or in a decade (6). In contrast, a case has been reported in which the attacks occurred daily (17). The onset has ranged from four to 35 years in one family (15), was consistently at 18 or 19 years of age in another (14) and, in yet another, the condition was evident in infants less than a year old (18). The attacks have been reported to cease in one family after the fourth decade (15).

Periodic paralysis has been observed in association with other diseases. One family was reported to have migraine as well as periodic paralysis (13) and another family with the trait had migraine and muscular atrophy that developed after years of attacks of paralysis (19). Others report the association of periodic paralysis with exophthalmic goiter (20–22). The transition of periodic paralysis to spinal muscular atrophy also has been described (23).

Many diverse clinical observations and even more diverse interpretations of these findings have been reported. Attacks have been thought to be precipitated by local application of cold (17), by glucose (5, 6, 24, 25), by water diuresis (26), and by insulin (2, 24, 25). In other cases, none of these was effective in bringing about an attack (10, 25, 26). Potassium chloride, thyroid extract (25), cholinergic drugs (26), and adrenal extract (2) have all been used effectively and ineffectively as therapeutic agents. Reports concerning potassium, sodium, phosphate, and magnesium ion levels have been conflicting (1, 2, 5, 7, 10, 22, 27, 28).

It has been postulated that the defect is related to the central nervous system, to the permeability of muscle cells, to hepatic or muscular glycogenesis or to a chronic deficit of potassium in the muscle (10, 28).

In addition to the analysis of the genetic pattern in this kindred we have made certain clinical and laboratory studies on some of these patients. We have studied methods of precipitating attacks, the effect of administering glucose or glucose and insulin, the effect of exercise and of enforced rest, and the correlation of these factors to serum potassium, sodium, magnesium, phosphate and blood glucose levels. Gross and microscopic examination of biopsy material from skeletal muscle as well as determination of its glycogen content also have been carried out.

## METHODS

Pedigree, genetic and clinical data were secured by interviews with the members of the kindred. The trait in this kindred is of such a nature that it is readily identified by the members of the families, and their reports of its occurrence have been consistently accurate in those patients whom we have examined. Where it was necessary to obtain information regarding individuals who are now dead, a check was made from as many different sources as possible in order to be sure that the data were accurate. Blood types and ability to taste for phenylthiocarbamide (PTC) were determined for use in linkage studies.

Five patients were studied in detail on the metabolic ward of the laboratory. A full history, physical examination and clinical laboratory investigation, as well as certain special determinations as summarized in the introduction, were carried out. Blood glucose values were determined by the method of Nelson (29) on alkaline zinc-barium filtrates of whole blood collected in a fluoride-oxalate anticoagulant. Serum potassium and sodium were measured on a flame photometer (30). Serum magnesium values were estimated by the colorimetric method of Kunkel and coworkers (31), muscle glycogen by the method described by Good and colleagues (32). The remaining chemical and hematologic methods used have been described in detail in other papers of this series and will not be reviewed here (33, 34).

## CLINICAL DESCRIPTION OF THE TRAIT

The clinical manifestations of the trait as observed in this kindred consisted of episodes of weakness or flaccid paralysis. Such attacks began and ended insidiously. They lasted from a few minutes to many hours or even several days in rare instances. In severe attacks all of the skeletal muscles were involved except those of facial expression, mastication, deglutition, speech, and respiration (diaphragm). Respiratory embarrassment of a severe character was not observed in any of these patients. In milder episodes localized areas were sometimes involved, usually the large muscles of the legs, arms or back, although an occasional individual had attacks in which only local involvement of the hands occurred. The pattern was usually symmetrical on both sides of the body. The paralysis was flaccid, deep tendon reflexes were absent and electrical excitability of the muscle was greatly reduced or lost.

The attacks began most frequently in the early morning and not infrequently were present on awakening. Activity tended to decrease the degree of weakness present if paralysis did not preclude movement. On the other hand, strenuous exercise which was followed by complete inactivity very frequently initiated an episode. This was the most reliable manner of initiating attacks in the patients whom we studied. Paralysis did not occur while the patient continued to be active but the more strenuous the activity and the more complete the ensuing rest, the more probable it was that a severe attack would result. The patients consistently reported that they could "fight off" an incipient attack by persistent activity. This, it may be noted, is the exact converse of the effect of activity and rest in myasthenia gravis.

A number of other factors also appeared to play a role in the precipitation of attacks. A heavy meal or, less frequently, the ingestion of a large amount of carbohydrate, particularly if taken before bed time, sometimes preceded an attack on the ensuing morning or day. No patient reported attacks precipitated by high carbohydrate intake during the preceding two hours.

Some patients reported no difficulty from food ingestion regardless of amount. Still other periods of recurrent severe episodes occurred without any apparent relation to the activities of the patient. These were just as severe as those having apparent relationship to preceding exercise or other factors which the patient regarded as responsible for the attack.

During the attacks there were no symptoms other than those which related to the weakness itself, except that during severe paralysis marked perspiration usually occurred. That this may precede the paralysis and possibly be a factor in its development was suggested by the drenching night sweats which frequently preceded morning attacks. One patient reported that profuse sweating preceded most of his severe attacks. On the other hand, the profuse perspiration induced by heavy work in hot weather does not induce paralysis unless it is followed by more or less complete inactivity.

The initial attacks occurred in the first year of life and the parents who were well acquainted with the disorder were able to recognize the presence of the trait regularly during the first months of life. The episodes tended to be most frequent during childhood and adolescence, gradually decreasing in severity and frequency with advancing age although they still occurred in aged individuals. There was a moderate degree of variability in the severity of the syndrome from patient to patient, some being only occasionally inconvenienced, others having attacks of such severity that they must miss work on the average of one day per week. In the individual patient as well, a long period during which little trouble was experienced might be followed by frequently recurrent attacks for periods lasting a few days or many months. Such periods were often associated with continued emotional stress.

On physical examination two observations could be made almost routinely even though the patient felt that he was normal at the time. A slight but definite degree of weakness was present in the extensors of the arms and legs which was sufficient to show up in sharp contrast to the good strength present in the flexors. These patients presented the appearance of muscular, hearty individuals, a fact which had caused certain of the male members no end of difficulty in dealing with the armed services where weakness in an apparently muscular individual is presumed to be evidence of laziness. The muscular appearance was particularly marked in the calf muscles and extensors of the arms, and was indistinguishable clinically from the pseudohypertrophy of childhood progressive muscular dystrophy, as previously discussed by one of us (35). Pathologically the findings are very different from those observed in the pseudohypertrophy of dystrophy, as will be described below.

The syndrome appears in both sexes. The symptoms are usually more severe in the males, possibly because of the somewhat more vigorous activity in which they engage and the relationship of exercise to the episodes of paralysis outlined above.

## LABORATORY STUDIES

No significant laboratory abnormality has been observed in the five male patients whom we have studied in detail except for the occurrence of high serum uric acid levels in two. No history suggestive of gouty arthritis was obtained on these nor on other normal or abnormal members of the group. Table I is a summary of the routine and certain special determinations which were performed. The fasting serum potassium values, taken at times when no significant degree of paralysis existed, were normal. Those presented are representative figures taken from a large series of determinations, none of which was abnormal. The normal creatinine excretion values are consistent with the apparently normal muscle mass. Creatine excretion was insignificant. Volumes of packed red cells ranging about 50 per cent correspond to normal male values for this area which is over 4,000 feet above sea level.

The results of some of our attempts to induce attacks by the administration of glucose or glucose and insulin are summarized in Table II. None of these was successful in producing a significant change in the degree of disability present at the start of the tests although serum potassium levels of about 3.1 meq./L were observed on several occasions.

Simultaneous urine collections showed a marked drop in the excretion of potassium, as is regularly observed during the hypopotassemia following carbohydrate or insulin administration. Thus the metabolic picture observed by Danowski has been reproduced without precipitating an attack of paralysis. The time relationships are shorter which possibly is a significant factor in the problem. No exactly similar experiments have been carried out in other cases of periodic paralysis as far as the authors are aware and we have not had an opportunity up to the present to duplicate the observation in a patient showing definite hypopotassemia during spontaneous attacks. It is noteworthy, however, that in nearly all of the previously reported patients in whom carbohydrate or insulin appeared to have been related to attacks, the paralysis developed after a delay of six or more hours and not at the time of maximum hypopotassemia. We observed no significant increase in attacks of paralysis in our patients during the 24 hours following these experiments.

Glucose tolerance was certainly not impaired in any of these tests. The rather low 30 and 60 min-

TABLE I

*Serum, whole blood and urine studies in hereditary periodic paralysis*

| | G. D. K.<br>10<br>Male | Bu. R.<br>19<br>Male | Bl. R.<br>22<br>Male | T. K. B.<br>23<br>Male | M. M. B.<br>27<br>Male |
|---|---|---|---|---|---|
| Potassium, meq./L | 5.7 | 4.2 | 4.0 | 4.2 | 4.0 |
| Sodium, meq./L | | 144 | 141 | 138 | 144 |
| Magnesium, meq./L | | | 2.0 | | 2.1 |
| Calcium, meq./L | | | | 5.3 | 5.5 |
| Phosphate, mg./100 ml. as P | | | 4.5 | 3.3 | 3.1 |
| $CO_2$ combining power, mM./L | | 25.9 | | 26.3 | 26.1 |
| Chloride, meq./L | 104 | 107 | | | 103 |
| Uric acid, mg./100 ml. | | 7.8 | 5.5 | 7.9 | 4.8 |
| Total protein, gms./100 ml. | | 8.9 | 7.5 | | 7.9 |
| Albumin, gms./100 ml. | | 6.6 | 5.2 | | 5.9 |
| Fasting blood sugar, mg./100 ml. | 78 | 70 | 72 | 72 | 64 |
| Blood urea nitrogen, mg./100 ml. | 10 | 12 | 15 | 14 | 12 |
| Routine clinical urine examination | *Normal* | *Normal* | *Normal* | *Normal* | *Normal* |
| Creatinine, mg./24 hrs. urine | 754 | 1,525 | 1,366 | | 1,459 |
| Creatine, mg./24 hrs. urine as creatinine | 70 | 200 | 0 | | 73 |
| Hct., % RBC | 42 | 52 | 54 | 49 | 47 |
| WBC in thousands/mm.$^3$ | 9.0 | 6.8 | 8.6 | 8.3 | 7.4 |

TABLE II

*Blood sugar, serum potassium and serum phosphate values after oral or I.V. administration of glucose with or without insulin*

| Patient and procedure | Time in minutes......... | 0 | 30 | 60 | 90 | 120 | 150 | 180 | 240 |
|---|---|---|---|---|---|---|---|---|---|
| T. K. B.<br>96 gms. glucose<br>I.V. | Blood glucose, mg./100 ml. | 74 | 234 | 48 | 39 | | 62 | | |
| | Serum potassium, meq./L | 4.2 | 4.1 | 4.1 | 4.1 | | 3.9 | | |
| | Serum sodium, meq./L | 140 | 134 | 137 | 138 | | 137 | | |
| | Serum phosphate, mg./100 ml. as P | 3.7 | 2.8 | 3.0 | 3.1 | | 3.8 | | |
| T. K. B.<br>100 gms. glucose<br>orally | Blood glucose, mg./100 ml. | 72 | 79 | 88 | 72 | 85 | | 68 | 82 |
| | Serum potassium, meq./L | 4.7 | 3.6 | 3.6 | 3.7 | 3.7 | | 4.2 | 4.0 |
| | Serum phosphate, mg./100 ml. as P | 4.2 | 3.5 | 3.5 | 3.6 | 3.7 | | 3.9 | 3.9 |
| M. M. B.<br>100 gms. glucose<br>I.V. | Blood glucose, mg./100 ml. | 53 | 207 | 164 | 76 | 34 | | 57 | 62 |
| | Serum potassium, meq./L | 4.8 | 4.0 | 3.5 | 3.5 | — | | 3.5 | 3.7 |
| | Serum phosphate, mg./100 ml. as P | 3.4 | 3.1 | 3.1 | 3.4 | 4.0 | | 4.3 | 4.4 |
| M. M. B.<br>200 gms. glucose<br>orally | Blood glucose, mg. % | 64 | 107 | 76 | 67 | 82 | | 72 | 77 |
| | Serum potassium, meq./L | 4.0 | 4.1 | 4.5 | 4.4 | 4.1 | | 3.7 | 4.4 |
| | Serum sodium, meq./L | 144 | 143 | 142 | 142 | 142 | | 141 | 139 |
| | Serum phosphate, mg./100 ml. | 3.6 | 3.2 | 3.1 | 3.2 | 3.3 | | 3.5 | 3.6 |
| Bl. R.<br>35 gms. glucose<br>I.V. | Blood glucose, mg./100 ml. | 72 | 170 | 52 | 54 | | | 74 | |
| | Serum potassium, meq./L | 3.8 | 3.4 | 3.7 | 3.9 | | | 4.3 | |
| | Serum phosphate, mg./100 ml. as P | 3.6 | 3.4 | 3.6 | 3.5 | | | 4.1 | |
| Bl. R.<br>100 gms. glucose and<br>25 units insulin I.V. | Blood glucose, mg./100 ml. | 106 | 141 | 86 | 80 | | 64 | | |
| | Serum potassium, meq./L | 3.9 | 3.6 | 3.1 | 3.4 | | 3.7 | | |
| | Serum magnesium, meq./L | 2.0 | 1.9 | 1.6 | 1.9 | | 1.9 | | |
| | Serum phosphate, mg./100 ml. as P | 4.6 | 3.9 | 3.6 | 3.8 | | 4.3 | | |
| Bu. R.<br>200 gms. glucose and<br>30 units insulin I.V. | Blood glucose, mg./100 ml. | 70 | 187 | 14 | 25 | 39 | 53 | 57 | |
| | Serum potassium, meq./L | 3.7 | 3.1 | 3.1 | 3.1 | 3.4 | 3.6 | 4.2 | |
| | Serum phosphate, mg./100 ml. as P | 5.0 | 3.2 | 3.4 | 3.3 | 3.8 | 4.3 | 4.7 | |

ute blood glucose levels after oral administration might suggest either impaired absorption or increased glucose tolerance. Intravenous glucose tolerance tests using one-half gram of glucose per kilogram of body weight showed normal curves in three patients and no other evidence of altered carbohydrate metabolism could be obtained. During episodes of mild and severe paralysis we have obtained repeated serum samples which have been analyzed for sodium, potassium, and magnesium, as well as a number of other substances. All of these values have been consistently normal. A few are presented in Table III; the values during attacks are those obtained at the time paralysis was most severe.

Additional comment about the serum potassium values seems in order because of the many previous observations of depressed levels. On two occasions we have obtained multiple samples at approximately 15 minute intervals during artificially induced attacks (exercise followed by enforced rest). The serum potassium values on these occasions varied over a narrow range (0.4 meq.) and showed no consistent pattern or correlation to the degree of paralysis at the time the serum was obtained. Electrocardiograms taken during the attacks showed no change from the control tracings.

We have observed no values below 3.5 meq./L except in association with the glucose-insulin infusions. All of the values were in the same range as observed in normal individuals in our laboratory.

Oral therapy with potassium chloride, either prophylactically or during an attack, failed to influence the course of the disorder. However, we have not had the opportunity to observe the effect of potassium given orally or intravenously during an episode of severe paralysis of more than about one hour's duration, and the possibility, albeit im-

TABLE III

*Serum electrolyte values before and at the time of maximum paralysis in four patients*

| Patient | Serum potassium meq./L. | | | Serum magnesium meq./L. | |
|---|---|---|---|---|---|
| | Before attack | During attack | Change | Control | During attack |
| G. D. K. | 5.7 | 5.7 | 0.0 | | |
| Bu. R. | 4.2 | 5.0 | +0.8 | | |
| Bl. R. | 3.9 | 3.7 | −0.2 | 2.0 | 2.0 |
| | 4.2 | 4.2 | 0.0 | | 1.95 |
| | 5.4 | 5.4 | 0.0 | | 2.2 |
| | 3.6 | 4.0 | +0.4 | | |
| | 3.8 | 4.2 | +0.4 | | |
| T. K. B. | 4.2 | 3.5 | −0.7 | | |

probable, exists that the effect might be different under such circumstances. Actually all of the group who have taken potassium prophylactically by mouth dislike the medication and report that they have experienced just as many and as severe attacks while receiving such therapy as at other times. In addition, a number have experienced nausea, dizziness, and a vague lack of well-being while taking potassium, and all have discontinued it within a few weeks of its first administration.

## PATHOLOGY

Three patients submitted to biopsy of their enlarged gastrocnemius muscle mass. Grossly the muscles appeared essentially normal, contracted on mechanical stimulation, and showed no evidence of fatty replacement or of fibrosis.

Microscopically, characteristic degenerative changes were recognized in the muscle of all three patients but the degree of change varied widely. In the sample which showed the slightest degree of alteration, most of the fibers were well preserved and their strial markings were clearly defined but their diameters exhibited greater than normal variation. Some fibers were unusually slender and were stained more strongly with eosin than were the fibers of normal size. Irregular clusters of nuclei appeared along the more slender portions and, in a few foci, appeared to replace the substance of fibers. In other places, portions of fibers were missing and the spaces were partly filled by pycnotic nuclei, monocytes, polymorphonuclear neutrophils, fibrocytes and a few well preserved muscle nuclei. The most characteristic feature was the presence of vacuoles near the centers of a few fibers and appearing at sites of fusiform enlargement (Figure 1). The largest of these were round, fusiform or oval, occupied the entire diameter of the fiber and were limited laterally only by sarcolemma. The smaller spaces appeared to arise within sites of basophilic degeneration where longitudinal and transverse striae were absent and acidophilic granules appeared. The vacuoles occurred as multiple foci of rarefaction, sometimes lined up along the axis of the fiber like a string of beads. The larger vacuoles, apparently formed by coalescence of several small ones, often contained acidophilic granules and occasionally were filled with faintly basophilic coarse granules. A few contained one or two pycnotic nuclei.

In the sample of muscle exhibiting the most advanced degree of degeneration, very few muscle fibers appeared normal although many retained their striae. Irregularly swollen, hyalinized fibers alternated with degenerated fibers containing vacuoles of all sizes and shapes. Areas of segmental disintegration were often associated with an increased number and pycnosis of nuclei. Some portions of fibers were represented by amorphous acidophilic debris.

Frozen sections stained with Sudan IV and hematoxylin failed to show abnormal quantities or distribution of lipids.

Sections of muscle, fixed in absolute alcohol and stained by Best's carmine method, showed marked irregularity of distribution of glycogen granules. Many coarse granules were found in those portions of the fibers which appeared swollen and degenerated. Some of the vacuoles contained amorphous masses and coarse granules which were carmine-positive but many of the vacuoles were entirely empty or contained unstained debris.

Two of the biopsy specimens were analyzed for total glycogen content. The values, together with those of two control specimens from human material, are listed in Table IV. It will be noted that all of the values fall within the normal range for muscle glycogen.

TABLE IV

*Glycogen content of muscle*

| Patient | Muscle | Glycogen as gms. glucose/100 gms. tissue |
|---|---|---|
| M. M. B. | Gastrocnemius | 1.01 |
| Bu. R. | Gastrocnemius | 0.46 |
| Control 1 | Rectus Abdominus | 1.33 |
| Control 2 | Rectus Abdominus | 0.76 |

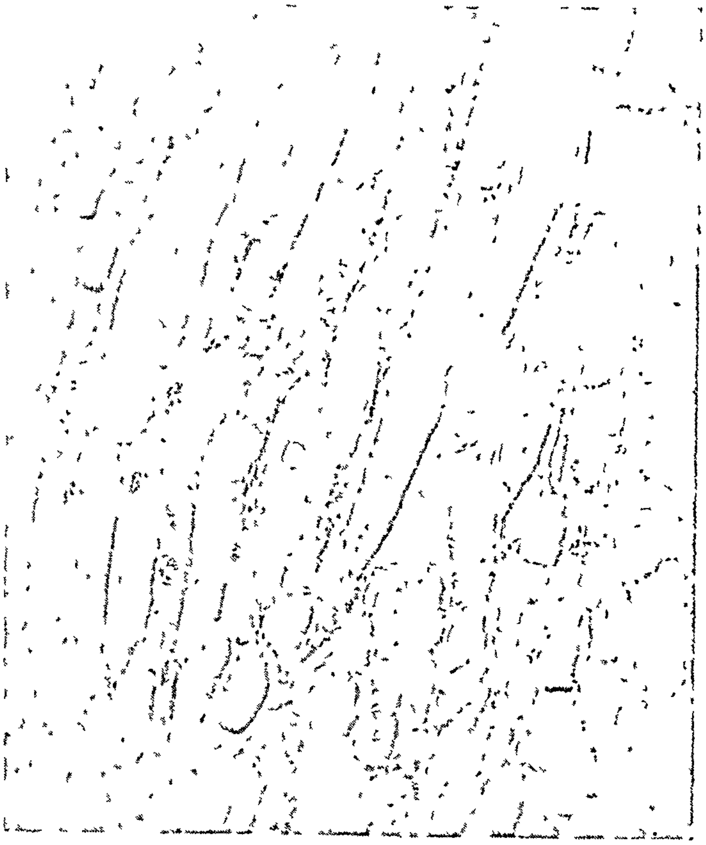

FIG. 1a.

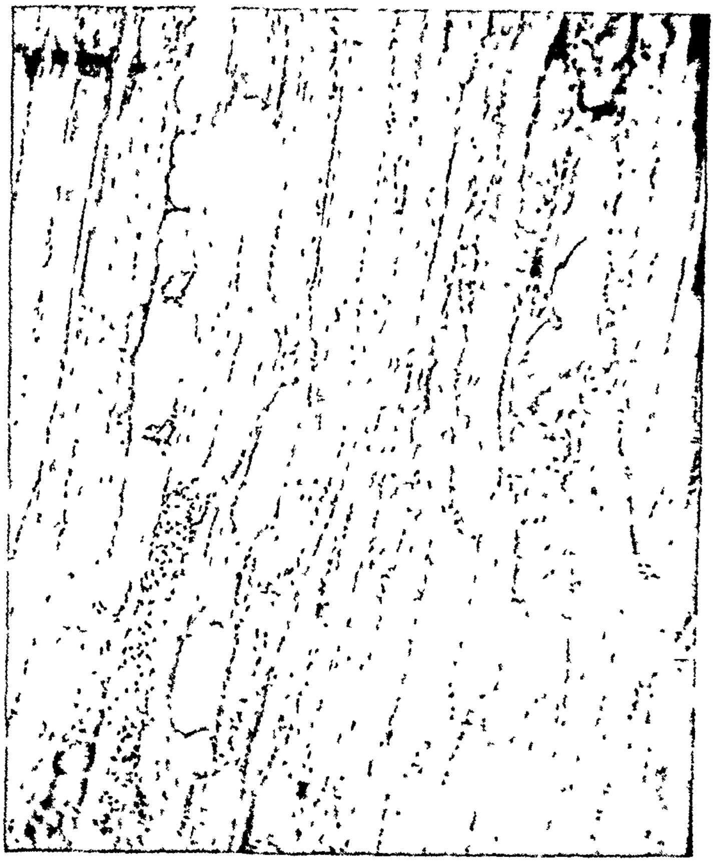

FIG 1c

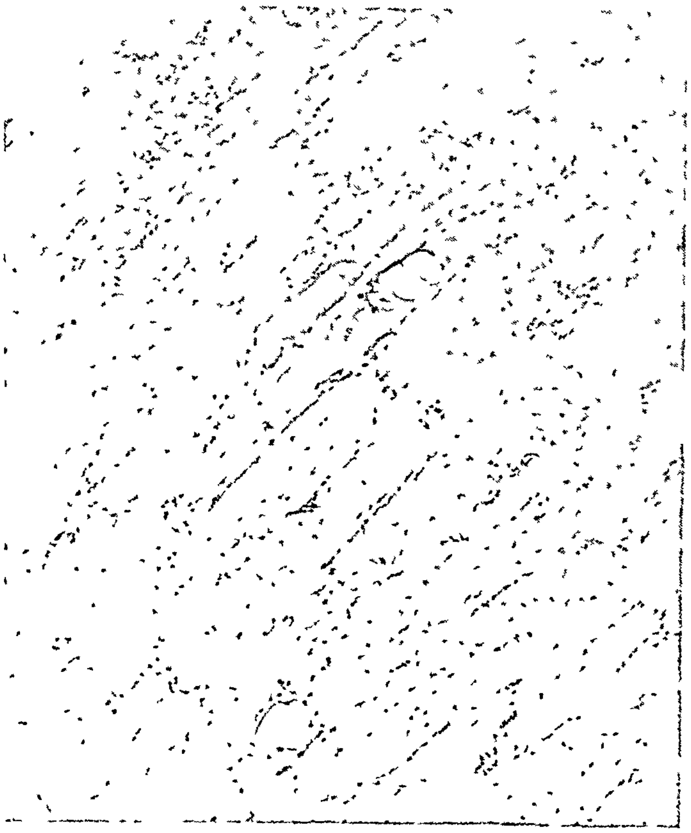

FIG 1b.

FIG 1 THREE PHOTOMICROGRAPHS ILLUSTRATING THE MICROSCOPIC CHANGES FOUND IN BIOPSY SPECIMENS FROM THE GASTROCNEMIUS MUSCLE

Note the central location of the vacuoles in (a) both in longitudinal and cross sections and the irregular size of the muscle fibers The more extensive changes in (b) and (c) are apparent, some fibers showing almost complete replacement The sections were stained with hematoxylin and eosin and are magnified about 78 diameters.

## FAMILY HISTORY

Figure 2 is a pedigree chart of the kindred reported in this study. I-1 and I-2 migrated to Utah in 1854. They had three daughters (II-1, 2, 3). II-1 died at the age of 13. III-8 and 9, daughters of II-3, state that their mother was affected with periodic paralysis and that she told them that her mother (I-1) and sister (II-2) were also affected. II-2 had five children all of whom are now dead. Interviews with their children, or with other members of the family who were in a position to know of their condition, indicated that none of the five children nor their descendants was affected with periodic paralysis. The condition of II-1 is not known.

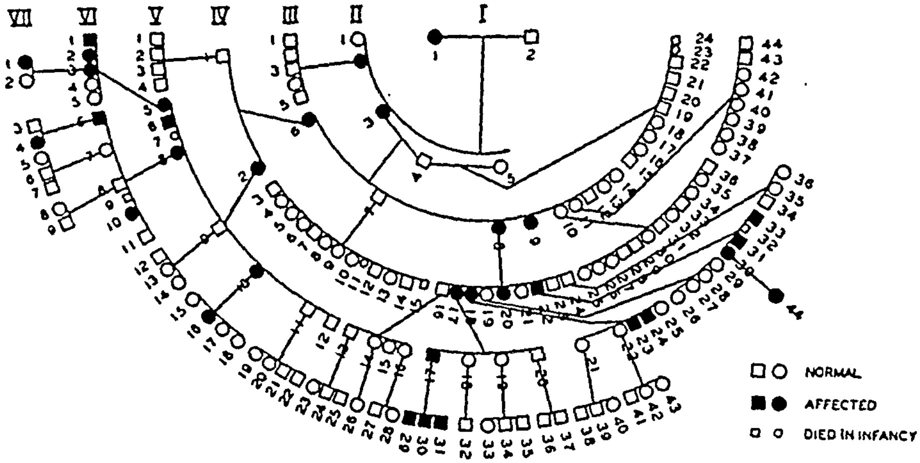

FIG. 2. PEDIGREE CHART OF KINDRED
Note that only affected individuals transmit the trait.

The husband of II-3 was a polygamist. He had ten children by his second wife (II-5) none of whom was affected with periodic paralysis. With the exception of I-1 and II-2, all of the known cases of periodic paralysis in this kindred descended from II-3. Nearly all members of the kindred are now living in Utah.

## INHERITANCE

Examination of Figure 2 shows that the trait is transmitted through one family line and that it never occurs in a child unless it has also occurred in a parent. Table V shows the numbers of normal and affected individuals by sex in the families in which the trait occurred. It includes all of the known offspring of individuals who have periodic paralysis in this kindred. Table VI compares the totals of Table V to the expected results if the trait is inherited as a dominant with complete penetrance.

TABLE V

*Segregation of children of 16 individuals affected with periodic paralysis*

| Family | | Affected | | Normal | | Condition unknown |
|---|---|---|---|---|---|---|
| Generation | Number | Male | Female | Male | Female | |
| I | 1 | — | 2 | — | — | 1 |
| II | 2 | — | — | 4 | 1 | |
| II | 3 | — | 3 | 2 | 4 | |
| III | 6 | — | 1 | 1 | — | |
| III | 8 | 1 | 3 | 3 | 2 | |
| IV | 2 | 1 | 3 | 3 | — | |
| IV | 17 | 1 | — | 1 | 2 | |
| IV | 18 | 2 | — | — | 3 | |
| IV | 22 | 2 | 1 | — | — | |
| V | 5 | 1 | 2 | — | 2 | |
| V | 8 | 1 | 1 | 2 | 1 | |
| V | 10 | — | 1 | — | 3 | |
| V | 17 | 3 | — | — | — | |
| V | 30 | — | 1 | — | — | |
| VI | 3 | — | 1 | — | 1 | |
| VI | 6 | — | 1 | 1 | — | |
| Totals | | 12 | 20 | 17 | 19 | 1 |

It is apparent that the trait occurs in approximately the same frequency in males and females and that approximately one-half of the children of an affected parent show the trait. This suggests that in this kindred, periodic paralysis is attributable to a simple dominant gene having complete penetrance. The deviation from the expected incidence is very slight. Since no two individuals showing the trait and no two relatives have married, affected individuals must be presumed to be heterozygous (Pp). The crosses, therefore, which have produced affected children, are back crosses (Pp × pp) and should produce one-half affected and one-half normal children. Since we have no homozygous individuals, there is no way of deter-

TABLE VI

*Statistical comparison of data on this kindred with the theoretic behavior of a dominant trait*

| | Observed | Calculated | Deviation | S.E. | D./S.E. |
|---|---|---|---|---|---|
| Affected | 32 | 34 | 2 | 4.123 | .485 |
| Normal | 36 | 34 | | | |

mining whether or not the dominance is complete. It is possible that the trait would be expressed differently in the homozygous condition.

Blood samples have been taken and determinations made for A, B, M, N and the various Rh antigens. Taste reactions to PTC were also determined. It was possible to obtain data from nearly all of the families segregating for paralysis who are now living. The data, however, were insufficient to warrant any conclusion regarding linkage relationships.

## EUGENIC IMPLICATIONS

In this kindred, periodic paralysis can be detected readily by members of the families concerned. Each individual showing the trait is heterozygous and stands an equal chance of transmitting or not transmitting it to each of his children. The trait can be eliminated only if each affected individual refrains from having children. Unaffected individuals do not transmit the trait.

## DISCUSSION

A number of unanswered problems remain concerning the disorder which we have observed in this group of people. Clinically the process is nearly identical with previously described cases of periodic paralysis whether hereditary or apparently sporadic. On the other hand, the failure to demonstrate any relationship of the attacks of paralysis to serum electrolyte concentrations, particularly the absence of hypopotassemia, sets the group apart from the best studied cases reported in the literature. Were it not for the defects which were discussed earlier, in the concept that serum potassium depletion is the fundamental mechanism leading to the paralysis in other cases, it might be concluded that we have been dealing with an entirely different disorder. This is hardly justified, however, in the light of the variability of the previously reported cases and the clinical indistinguishability of this syndrome from them. It is possible that all types of periodic paralysis are due to a single pathogenetic mechanism the nature of which has not been identified. However, the evident hypopotassemia seen in other patients makes this rather improbable, and therefore it is more likely that there are two or more types of periodic paralysis.

The frequently reported relationship of attacks to carbohydrate ingestion led us to postulate the possibility of some abnormality in the immediate availability of glycogen in muscle for the production of energy. Our studies to date have yielded no evidence to confirm such a theory; instead they have only served to emphasize the poor relationship between carbohydrate administration and attacks in this kindred.

The unusual pathologic picture of vacuolization of muscle fibers has been observed before, as far as we are aware, only in acute traumatic or infectious injury of muscle or in experimental obstruction of the deep lymphatics. The lack of inflammatory cells or evidence either clinically or pathologically of lymph stasis in these patients is not consistent with such an explanation here, however. A somewhat similar but more severe lesion has been observed in glycogen storage (von Gierke's) disease in children. The possibility that the disorder which we have described represents a related phenomenon in which only the muscle was involved seemed reasonable. However, our inability to demonstrate excess total glycogen or microscopic distribution of glycogen in the muscle fiber characteristic of that disorder makes this highly improbable.

The clean-cut genetic pattern of dominant inheritance with complete penetrance which we have found is in contrast to the findings in some of the previously reported families. This would appear to be additional evidence of the multiple character of the genetic mechanisms which result in the clinical syndrome of periodic paralysis. However, as we have emphasized previously (36), it is difficult to obtain reliable genetic data unless many of the normal and abnormal individuals are examined. In many published reports this was not done.

## CONCLUSIONS

1. A study of periodic paralysis occurring in 33 individuals in seven generations of a single kindred is reported.

2. Clinically the disorder begins in infancy with recurrent attacks of weakness or flaccid paralysis. Some extensor weakness and enlargement of muscles is evident at all times.

3. The attacks are most consistently produced by vigorous exercise *followed* by complete rest.

4. No correlation of the attacks with the serum potassium or other electrolyte concentrations could be established.

5. An unusual pathologic picture of vacuolization of scattered muscle fibers was found in biopsy material. Neither fat nor glycogen could be demonstrated in the vacuoles and it is presumed that they contained only edema fluid.

6. The pattern of inheritance is that characteristic of a simple mendelian dominant trait with complete penetrance.

## ACKNOWLEDGMENTS

We are deeply indebted to Dr. Virginia Davenport and Dr. Harold Brown for certain of the electrolyte determinations.

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# RESPIRATORY REGULATION DURING EXERCISE IN UNCONDITIONED SUBJECTS[1]

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(Submitted for publication December 13, 1950; accepted, March 12, 1951)

## INTRODUCTION

The regulation of breathing during muscular exercise has been the subject of much work and speculation. Most of the experience has been gained with subjects in good physical training. In general, blood chemical changes in these subjects have not been great during exercise, and it has been difficult to account for the amount of ventilation on the basis of these changes and other known respiratory stimulants. The present study was done on untrained subjects in only fair physical condition. It seemed likely that the coordination of various respiratory drives would be less smooth in untrained subjects than in trained persons, so that the part played by different stimuli might become more obvious. The conduct of such studies has been simplified by the recent development of techniques for recurrent sampling of arterial blood during the course of exercise. Nearly all past studies have had to rely on indirect means of estimating arterial blood changes.

## METHODS

The subjects were 11 male physicians and medical students in normal health but not in physical training. They ranged in age from 22 to 35 years and in surface area from 1.86 to 2.12 sq. m. They were not fasting and came to the experiment directly from their work. Arterial blood samples were taken from the brachial artery through an in-lying Cournand needle attached by flexible plastic tubing to a series of three way stopcocks. Blood was taken into syringes wet with heparin. For pH determinations, sodium fluoride was added as a preservative. The syringes were capped and stored in ice water. Analyses were completed within two to four hours. The subjects breathed through a Douglas valve into a recording spirometer. For determination of oxygen consumption the expired air was diverted into a Douglas bag. Gas mixtures other than air were supplied to the inspiratory side of the system from large Douglas bags. These mixtures consisted of 15% $O_2$ in $N_2$, tank oxygen (99.5%), and 5% $CO_2$, 21% $O_2$ in $N_2$. Resting measurements were made with the subject seated. Ventilation was also measured with the subject standing, just before exercise. Exercise was carried out in an airconditioned room at 25° C. on a treadmill at a speed of 5.1 miles per hour on a 3.5% grade. For most of the subjects this exercise rate was fairly severe. Oxygen consumption, measured in eight subjects about five minutes after beginning exercise, was $2{,}290 \pm 90$ cc./min. (range: $1{,}760 - 2{,}610$). Ventilation was recorded continuously and is expressed as ventilation at room temperature ($25 \pm 1°$ C.).[3] Arterial blood samples were taken at points during the breathing of various gas mixtures, at rest and exercise, when it became evident from the respiratory tracing that the ventilation rate had been nearly steady for at least 30 seconds. The subjects were studied on two separate days, one of which was devoted to the effects of the carbon dioxide mixture, and the other to the effects of the various oxygen mixtures.

Respiratory gas analyses were done with the Haldane apparatus or with a Pauling oxygen meter. Arterial blood oxygen content and per cent oxygen saturation were determined by a spectrophotometric method (1). The pH of whole blood was measured with a Cambridge Model R pH meter equipped with an inclosed glass electrode. Measurements were made at room temperature, usually 25–26° C., and the result was corrected to 37° C. by Rosenthal's factor (2). This temperature was arbitrarily chosen for all pH measurements on arterial blood. pH determinations were made in duplicate in nearly every case, and duplicate determinations agreed to within 0.02 pH units or less. The stability of the instrument was checked with a pH 4.00 acid phthalate buffer or a pH 7.01 phosphate buffer between each reading. The carbon dioxide content of whole blood was determined by the method of Van Slyke and Neill (3), and the plasma carbon dioxide content was estimated from this value and from the pH and hemoglobin by the line chart of Van Slyke and Sendroy (4). From the pH and $CO_2$ content of arterial plasma at 37° C., the $CO_2$ tension of arterial blood was calculated by the Henderson-Hasselbalch equation. For the calculation of $CO_2$ tension at 37° C., pK′ was taken to be 6.11, as found by Dill, Daly and Forbes (5). Where the method was evaluated, as described below, by equilibration of blood in a tonometer at 38° C., a pK′ value of 6.10 was used, as determined for this temperature by Hastings, Sendroy, and Van Slyke (6).

The determination of $CO_2$ tension by this indirect means

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[1] This work was supported by grants from the Life Insurance Medical Research Fund and the Anna H. Hanes Memorial Fund.

[2] U. S. Public Health Service Postdoctorate Research Fellow.

[3] Range of barometric pressure: 747–757 mm. Hg.

depends upon several measurements and calculations, of which pH determination and temperature correction seem most liable to error. The validity of the procedure was therefore tested by equilibrating blood samples in tonometers with a variety of $CO_2$ tensions at 38° C. The actual $CO_2$ tension or "direct tension" was determined by Haldane analysis of the gas mixture in the tonometer. The blood was then removed from the tonometer, cooled to room temperature, and determination of "indirect $CO_2$ tension" carried out as described above, except that pH's were corrected to 38° C. In this way, 23 comparisons were made between direct and indirect $CO_2$ tensions. The direct tensions ranged from 19.5 to 58.1 mm. Hg, and the corrected direct pH's from 7.59 to 7.13. The per cent oxygen saturation of these bloods was between 70 and 100% in 13 instances, and between 25 and 70% in the remainder. The mean indirect $CO_2$ tension exceeded the direct by 1.62 mm. Hg, with a standard deviation of 2.03, and a standard error of 0.42. This difference, though small, was significant ($P < .01$). In addition, a comparison of indirect arterial $CO_2$ tension with alveolar $CO_2$ tension was made in 17 instances. The alveolar airs were collected during rest and exercise with the variations in technique for these conditions described by Dill and colleagues (7, 8), and also during hyperventilation on air and various $CO_2$ mixtures. Alveolar airs do not provide a satisfactorily exact check on arterial $CO_2$ tensions, but the comparison was thought to be required by the possibility that changes in the blood during strenuous exercise might seriously invalidate the corrections made in the indirect measurement of $CO_2$ tension. The alveolar tensions ranged from 17.9 to 76.7 mm. Hg, and the blood pH's from 7.59 to 7.15. The mean indirect arterial $CO_2$ tension exceeded the alveolar tension by 1.6 mm. Hg, with a standard deviation of 3.7. The difference was not significant. These agreements seem satisfactory. It is not clear why the indirect $CO_2$ tension is significantly greater than the direct. It seems most probable that the error lies in measurement of blood pH. The difference could be caused by a mean error of approximately .02 units below the true value. The deviations between direct and indirect $CO_2$ tensions were not more marked at extreme pH ranges than at more ordinary values. This is of importance because Rosenthal's temperature coefficient for whole blood was established for a pH range of 7.25 to 7.45, while the temperature coefficient of serum has been found to vary considerably at extreme pH ranges (6.6 and 7.8) (5). The present results indicate that Rosenthal's coefficient for human whole blood can satisfactorily be extended over the pH ranges encountered in this study.

## RESULTS

The data on each subject relating respiratory volume to arterial blood measurements at rest and exercise and during the breathing of various gas mixtures are presented in Tables I and II.

### *Resting arterial blood*

The mean values for resting arterial blood are presented in Table III. Of the 11 subjects, arterial blood was obtained from one on only a single occasion, and from two subjects on three occasions. In the remaining eight, samples were taken during two separate trials. In determining mean values each subject was given equal weight. The arterial oxygen saturation is within the general range of recent reports by Comroe and Walker (97.5%) (9), Wood and Geraci (97.9%) (10), and Drabkin and Schmidt (98.6%) (11). The pH is somewhat lower than that found for arterial blood by Dill, Edwards, and Consolazio (12) (7.40) and by Dill and associates (7.39) (13), but higher than that reported by Barr, Himwich, and Green (7.30–7.36) (14). The $CO_2$ tension is slightly higher than that reported by Dill and colleagues (41.0, 40.9) (12, 13), higher than that of Lilienthal and coworkers, by Riley's direct method (37.8) (15), close to that of Galdston and Wollack (42 direct, 44 indirect) (16), and less than that of Barr and colleagues (44–45) (14). As noted above, the mean $CO_2$ tension is probably about 2 mm. Hg too high, and the pH .02 units too low. In addition, the values are probably affected by the small dead space of the respiratory apparatus, and are almost certainly affected by the fact that the subjects were not in a basal state, but came to the experiment directly from their daily activities. Under these conditions the variability in pH and $CO_2$ tension among normal subjects was quite striking.

### *Arterial blood changes during exercise*

The present observations are not directly comparable to many of those reported in the literature for the reason that the exercise period was too short in most cases for the subject to reach a steady state. In addition, the subjects were untrained, and the exercise accordingly varied in its relative severity. For some subjects it was severe enough to preclude reaching a steady state.

The arterial blood oxygen capacity rose in all subjects, the mean increase being 6%. This is within the general range previously reported (8, 14, 17–19). The increase had reached nearly its full value after three minutes of exercise. The per cent oxygen saturation gradually declined dur-

TABLE I

*Effect of 5% $CO_2$ during exercise*

| Subject, Surface area | State | Inspired mixture | Time on mixture | Ventilation | % Arterial $O_2$ saturation | Arterial serum $CO_2$ | Arterial pH | Arterial $CO_2$ tension |
|---|---|---|---|---|---|---|---|---|
| (*sq. m.*) | | | | (*l./min., 25° C.*) | | (*vol. %*) | | (*mm. Hg*) |
| R. | Rest | Air | | 6.1 | 95.8 | 53.2 | 7.41 | 38 |
| 1.93 | | 5% $CO_2$ | 2:00 | 9.6 | 98.4 | 57.1 | 7.34 | 47 |
| | Exercise | Air | 3:25 | 51.4 | 95.9 | 42.9 | 7.26 | 37 |
| | | 5% $CO_2$ | 1:38 | 81.8 | 95.8 | 46.2 | 7.19 | 53 |
| | | Air | 1:50 | 78.6 | 94.2 | 34.5 | 7.20 | 39 |
| R. | Rest | Air | | 6.4 | 97.5 | 53.5 | 7.38 | 41 |
| 1.86 | | 5% $CO_2$ | 1:50 | 22.6 | 99.6 | 56.2 | 7.32 | 49 |
| | Exercise | Air | 2:45 | 71.1 | 98.8 | 40.7 | 7.29 | 38 |
| | | 5% $CO_2$ | 1:10 | 122.0 | 98.2 | 47.7 | 7.13 | 62 |
| | | Air | 0:54 | 84.0 | 97.8 | 34.5 | 7.21 | 38 |
| M. | Rest | Air | | 9.7 | 99.3 | 51.0 | 7.38 | 39 |
| 1.90 | | 5% $CO_2$ | 2:00 | 11.5 | 98.7 | 54.4 | 7.29 | 50 |
| | Exercise | Air | 3:50 | 58.0 | 97.1 | 43.3 | 7.20 | 49 |
| | | 5% $CO_2$ | 1:27 | 87.0 | 97.4 | 49.6 | 7.13 | 65 |
| | | Air | 1:00 | 67.0 | 97.5 | 40.3 | 7.23 | 42 |
| K. | Rest | Air | | 6.5 | 98.2 | 58.1 | 7.38 | 44 |
| 1.88 | | 5% $CO_2$ | 2:00 | 17.5 | 99.7 | 60.1 | 7.32 | 52 |
| | Exercise | Air | 4:00 | 40.3 | 97.8 | 48.5 | 7.29 | 45 |
| | | 5% $CO_2$ | 2:00 | 72.7 | 99.1 | 53.1 | 7.19 | 61 |
| | | Air | 2:10 | 51.1 | 97.8 | 45.1 | 7.27 | 43 |
| D. | Rest | Air | | 15.9 | 99.4 | 52.6 | 7.50 | 31 |
| 2.12 | | 5% $CO_2$ | 1:50 | 16.8 | 99.2 | 59.5 | 7.34 | 49 |
| | Exercise | Air | 3:40 | 49.4 | 95.1 | 51.9 | 7.29 | 48 |
| | | 5% $CO_2$ | 1:00 | 66.0 | 96.8 | 55.9 | 7.14 | 71 |
| | | Air | 2:10 | 65.0 | 94.0 | 44.6 | 7.21 | 49 |
| L. | Rest | Air | | 8.3 | 98.4 | 56.7 | 7.35 | 46 |
| 1.95 | | 5% $CO_2$ | 2:00 | 15.2 | 98.7 | 61.3 | 7.25 | 62 |
| | Exercise | Air | 3:45 | 42.0 | 97.0 | 51.5 | 7.20 | 58 |
| | | 5% $CO_2$ | 1:15 | 57.0 | 97.5 | 53.2 | 7.10 | 73 |
| | | Air | 1:50 | 59.7 | 95.6 | 42.8 | 7.13 | 56 |
| C. | Rest | Air | | 6.3 | 98.4 | 56.4 | 7.36 | 45 |
| 1.92 | | 5% $CO_2$ | 1:50 | 10.7 | 98.8 | 60.6 | 7.29 | 56 |
| | Exercise | Air | 3:30 | 44.3 | 98.3 | 53.2 | 7.31 | 47 |
| | | 5% $CO_2$ | 1:45 | 81.5 | 98.5 | 57.6 | 7.22 | 62 |
| | | Air | 1:00 | 51.0 | 97.8 | 50.0 | 7.32 | 43 |
| | Rest | Air | | 7.6 | 97.4 | 55.7 | 7.35 | 45 |
| | | 5% $CO_2$ | 2:45 | 17.6 | 99.2 | 59.9 | 7.31 | 53 |
| G. | Rest | Air | | 9.1 | 98.9 | 60.7 | 7.40 | 44 |
| 2.04 | | 5% $CO_2$ | 1:45 | 14.0 | 99.1 | 63.1 | 7.33 | 53 |
| | Exercise | Air | 3:15 | 47.0 | 96.9 | 52.7 | 7.28 | 50 |
| | | 5% $CO_2$ | 1:00 | 58.0 | 97.5 | 58.7 | 7.19 | 67 |
| | | Air | 1:10 | 52.4 | 96.1 | 53.0 | 7.27 | 51 |
| S. | Rest | Air | | 7.1 | 97.8 | 56.4 | 7.37 | 44 |
| 1.89 | | 5% $CO_2$ | 1:50 | 22.2 | 98.9 | 59.2 | 7.32 | 51 |
| | Exercise | Air | 3:27 | 37.4 | 97.3 | 52.7 | 7.34 | 43 |
| | | 5% $CO_2$ | 1:20 | 72.5 | 97.6 | 59.9 | 7.20 | 67 |
| | | Air | 1:03 | 49.9 | 97.4 | 51.4 | 7.27 | 49 |
| T. | Rest | Air | | 6.9 | 96.7 | 57.5 | 7.36 | 46 |
| 2.04 | | 5% $CO_2$ | 1:45 | 11.8 | 98.9 | 61.8 | 7.29 | 57 |
| | Exercise | Air | 3:15 | 30.0 | 96.8 | 58.5 | 7.29 | 54 |
| | | 5% $CO_2$ | 1:02 | 27.3 | 97.2 | 64.0 | 7.19 | 73 |
| | | Air | 1:50 | 31.0 | 98.1 | 57.8 | 7.29 | 53 |
| | Rest | Air | | 5.9 | 97.7 | 58.6 | 7.36 | 47 |
| | Exercise | Air | 4:10 | 20.5 | 93.1 | 57.8 | 7.23 | 61 |
| | | 5% $CO_2$ | 2:05 | 34.8 | 97.3 | 60.6 | 7.15 | 75 |
| | | Air | 2:46 | 36.0 | 95.8 | 53.6 | 7.22 | 58 |

TABLE II

*Effect of 15% $O_2$ and tank $O_2$ during exercise*

| Subject | State | Inspired mixture | Time on mixture | Ventilation | % Arterial $O_2$ saturation | Arterial serum $CO_2$ | Arterial pH | Arterial $CO_2$ tension |
|---|---|---|---|---|---|---|---|---|
| | | | | (l./min., 25° C.) | | (vol. %) | | (mm. Hg) |
| H. | Rest | Air | | 8.9 | 99.4 | 51.6 | 7.38 | 39 |
| | Exercise | Air | 2:50 | 66.6 | 97.7 | 43.5 | 7.28 | 41 |
| | | 15% $O_2$ | 0:54 | 94.8 | 86.9 | 37.2 | 7.27 | 36 |
| | | Air | 1:25 | 87.4 | 95.4 | 33.1 | 7.15 | 41 |
| | *Exercise | Air | 8:00 | 84 | | | | |
| | | 100% $O_2$ | 1:15 | 70 | | | | |
| R. | Rest | Air | | 7.6 | 97.6 | 55.0 | 7.37 | 43 |
| | Exercise | Air | 3:00 | 72.0 | 98.0 | 43.4 | 7.23 | 46 |
| | | 15% $O_2$ | 1:00 | 97.0 | 86.9 | 38.1 | 7.21 | 42 |
| | | Air | 1:10 | 72.0 | 95.4 | 36.5 | 7.16 | 45 |
| | | 100% $O_2$ | 1:30 | 63.0 | 100.0 | 36.4 | 7.18 | 43 |
| M. | Rest | Air | | 12.7 | 99.2 | 52.1 | 7.44 | 35 |
| | Exercise | Air | 2:40 | 65.0 | 98.0 | 44.4 | 7.32 | 38 |
| | | 15% $O_2$ | 1:24 | 84.0 | 89.4 | 39.4 | 7.31 | 35 |
| | | Air | 1:10 | 73.0 | 97.3 | 40.7 | 7.25 | 41 |
| | | 100% $O_2$ | 1:10 | 49.0 | 100.0 | 38.2 | 7.20 | 43 |
| K. | Rest | Air | | 8.3 | 99.4 | 54.6 | 7.32 | 47 |
| | Exercise | Air | 1:36 | 69.1 | 99.1 | 48.8 | 7.27 | 47 |
| | | 15% $O_2$ | 1:15 | 63.0 | 86.7 | 41.8 | 7.25 | 42 |
| | | 100% $O_2$ | 1:24 | 64.0 | 100.0 | 40.0 | 7.19 | 46 |
| | | Air | 1:15 | 70.6 | 94.5 | 38.4 | 7.23 | 40 |
| D. | Rest | Air | | 13.9 | 98.7 | 51.3 | 7.42 | 36 |
| | Exercise | Air | 3:36 | 40.7 | 96.2 | 50.5 | 7.23 | 53 |
| | | 15% $O_2$ | 1:20 | 61.2 | 78.0 | 46.5 | 7.22 | 54 |
| | | Air | 1:15 | 53.5 | 95.8 | 43.7 | 7.17 | 52 |
| | | 100% $O_2$ | 1:27 | 49.9 | 100.0 | 44.5 | 7.14 | 57 |
| L. | Rest | Air | | 6.6 | 98.3 | 57.9 | 7.33 | 49 |
| | Exercise | Air | 3:36 | 40.0 | 97.1 | 52.6 | 7.26 | 52 |
| | | 15% $O_2$ | 1:46 | 40.0 | 88.7 | 49.8 | 7.24 | 51 |
| | | Air | 1:57 | 48.0 | 96.0 | 49.2 | 7.21 | 54 |
| | | 100% $O_2$ | 1:46 | 43.0 | 100.0 | 48.6 | 7.15 | 61 |
| | | Air | 1:45 | 54.0 | 95.0 | 46.9 | 7.14 | 60 |
| C. | Rest | Air | | 7.8 | 98.0 | 56.3 | 7.29 | 52 |
| | Exercise | Air | 2:36 | 40.6 | 97.9 | 50.9 | 7.29 | 47 |
| | | 15% $O_2$ | 1:36 | 56.5 | 90.4 | 47.9 | 7.26 | 47 |
| | | 100% $O_2$ | 2:00 | 38.8 | 100.0 | 54.7 | 7.30 | 49 |
| | | Air | 1:54 | 51.0 | 97.3 | 49.4 | 7.32 | 43 |
| B. | Rest | Air | | 7.4 | 99.7 | 54.0 | 7.32 | 47 |
| | Exercise | Air | 3:10 | 37.2 | 96.6 | 50.8 | 7.24 | 52 |
| | | 15% $O_2$ | 1:36 | 53.0 | 88.4 | 49.2 | 7.26 | 48 |
| | | 100% $O_2$ | 2:25 | 40.6 | 100.0 | 49.4 | 7.24 | 51 |
| | | Air | 2:05 | 47.0 | 96.5 | 48.8 | 7.29 | 45 |
| G. | Rest | Air | | 9.6 | 99.0 | 57.4 | 7.38 | 44 |
| | Exercise | Air | 3:51 | 37.6 | 97.8 | 54.0 | 7.34 | 45 |
| | | 15% $O_2$ | 1:40 | 46.0 | 89.3 | 53.4 | 7.36 | 42 |
| | | Air | 2:18 | 40.5 | 97.1 | 52.6 | 7.31 | 47 |
| | | 100% $O_2$ | 1:39 | 35.0 | 100.0 | 53.1 | 7.29 | 49 |
| | | Air | 1:57 | 40.9 | 96.7 | 53.2 | 7.31 | 47 |
| S. | Rest | Air | | 8.8 | 95.3 | 59.3 | 7.33 | 50 |
| | Exercise | Air | 2:20 | 36.6 | 95.3 | 55.5 | 7.20 | 62 |
| | | 15% $O_2$ | 2:10 | 50.9 | 84.4 | 49.8 | 7.23 | 52 |
| | | 100% $O_2$ | 2:40 | 32.4 | 100.0 | 54.1 | 7.18 | 63 |
| | | Air | 2:36 | 29.7 | 93.0 | 56.7 | 7.20 | 63 |
| T. | Rest | Air | | 9.9 | 98.1 | 58.6 | 7.35 | 47 |
| | Exercise | Air | 2:36 | 31.4 | 97.6 | 55.2 | 7.28 | 52 |
| | | 15% $O_2$ | 1:50 | 31.4 | 73.6 | 54.3 | 7.23 | 57 |
| | | 100% $O_2$ | 2:00 | 36.3 | 100.0 | 51.8 | 7.24 | 53 |
| | | Air | 1:20 | 36.6 | 95.3 | 50.0 | 7.20 | 56 |

* Separate study.

TABLE III

*Mean arterial blood values of 11 subjects at rest and during exercise*

| State | Ventilation | Blood $CO_2$ content | Serum $CO_2$ content | pH | $CO_2$ tension | $O_2$ capacity | % $O_2$ saturation |
|---|---|---|---|---|---|---|---|
| | *(l./min., 25° C.)* | *(vol. %)* | *(vol. %)* | | *(mm. Hg)* | *(vol. %)* | |
| Rest | 8.7±0.7 | 46.3±0.7 | 55.4±0.8 | 7.37±.01 | 43.3±1.5 | 19.51±0.32 | 98.3 |
| Exercise 3'11"* | 47.1±4.0 | 41.8±1.2 | 49.9±1.5 | 7.27±.01 | 48.2±1.6 | 20.52±0.37 | 97.1 |
| Exercise 8'20"* | 57.2±4.6 | 38.1±1.7 | 45.4±2.1 | 7.22±.02 | 47.6±2.1 | 20.67±0.33 | 96.1 |

* Average time. *(Ranges: 1'36" to 4'30" and 5'45" to 11'30".)* Indicated range following mean values denotes standard error.

ing the exercise period, falling by an average of 2.2%. A similar fall has been reported by Asmussen and Nielsen (20). The arterial oxygen tension has been found to fall during exercise (15). In addition, the pH fall in our subjects would have the effect of shifting the oxyhemoglobin dissociation curve so that the per cent saturation would be less for a given oxygen tension.

For the group as a whole there was a considerable fall in pH and in serum $CO_2$ content. A large change had occurred by the time of the first sample, and the decline was continued in the second sample. Decreases of arterial pH and $CO_2$ content during exercise are a reflection of the accumulation of lactic acid (8, 21), and changes of this magnitude indicate that the rate of exercise was severe for the group as a whole. With subjects in better training, exercise rates of this degree or much higher may be maintained without the accumulation of large amounts of lactic acid and without a decline in pH (8, 22). However, with exercise which is severe for the subject, fall in arterial pH and serum $CO_2$ content of this general magnitude have been reported (14, 21, 23). Our experience is similar to Barr's and Himwich's (23) in the finding that resting pH and $CO_2$ content may not be restored for many minutes after exercise.

The mean arterial $CO_2$ tension was slightly increased during exercise. Because of the great individual variation, the difference between the resting and exercising levels is not significant for the group as a whole. Nielsen (22) reported that the $CO_2$ tension falls during exercise and that the fall is greater with increasing severity of exercise. His observations were made during the steady state of exercise on two highly trained subjects, and the arterial $CO_2$ tension was calculated from the composition of expired air and the respiratory dead space. Data on this subject are relatively few. The observations of Barr and associates (14), and of Galdston and Wollack (16) showing a fall or no substantial change in arterial $CO_2$ tension were made on arterial blood drawn shortly after exercise was completed. Lilienthal and colleagues (15), measured $CO_2$ tension by Riley's method in arterial blood drawn during exercise from six subjects. The sampling time was comparable to that of the present study, but, judging from the oxygen consumption, the severity of exercise was somewhat less. They found an average increase of approximately 3 mm. Hg in arterial $CO_2$ tension during work. Dill and associates have investigated the arterial $CO_2$ tension during exercise as measured by "inspiratory" alveolar air. There was considerable individual variation, but the average tension, after a steady state was reached, was not far from the resting value (7, 8). Four trained subjects at graded work rates showed a moderate increase in $CO_2$ tension with lighter work rates and a gradual decline toward the resting level as the rate was increased (13). Briggs (24) studied subjects under a very large work load. In many of the best performers, there was a high $CO_2$ content in the expired air (up to 8% in two subjects), indicating a very high arterial $CO_2$ tension. In short, the available evidence indicates that the arterial $CO_2$ tension during exercise may rise, fall, or remain nearly unchanged depending upon the individual subject, the work rate, and the duration of work. In four cases in the present study the $CO_2$ tension within one minute after exercise was found to be substantially the same as the original resting value.

### *The course of ventilation during exercise*

At the start of exercise, as found by Krogh and Lindhard (25), there is an immediate increase in ventilation. With our subjects, this increased ventilatory rate was maintained with relatively minor fluctuations, particularly in the first four to five breaths, for roughly a minute. A rather sharp further increase in ventilation then occurred. This sharp "secondary rise" in ventilation was present consistently except in one subject, T., in whom it appeared in only one of three trials. The secondary rise usually took place within a period of one to three breaths. The time at which the secondary rise occurred varied from 30 to 67 seconds after the onset of exercise, with an average time of 52 seconds. The mean increase at this point was 10.4 ± 1.6 l./min. The ventilation thereafter rose much more gradually and smoothly, reaching a steady state in some cases. This secondary rise seems not to have been commented upon before, although close observations of breathing during early exercise have been made by Krogh and Lindhard (25) and by Asmussen and Nielsen (26). Their subjects were in good physical condition. Continuous recordings of ventilation throughout the course of exercise have been made by Rahn and Otis (27) and by Bruce and coworkers (28), without the finding of a sharp break in ventilation. In these studies the subjects were untrained, but the work rate was quite mild.

It seemed possible that the secondary rise might reflect the beginning of arterial blood changes resulting from exercise. This possibility was examined in two further subjects who showed a well-marked secondary rise on moderate exercise (corresponding to an $O_2$ consumption of about 1,400 cc./min.). The results are presented in Table IV. It is apparent that there was a rapid fall in pH and a rise in arterial $CO_2$ tension during early exercise. The total extent of these changes, as measured by the effect on ventilation of breathing $CO_2$ at rest, could account roughly for the magnitude of the secondary rise. However, the ventilation rise is sharp, while the blood chemical changes are more gradual. This difference may reflect a lag between arterial blood changes and the ventilatory response. It seems possible only to conclude that chemical changes may occur quite early in the arterial blood of untrained subjects even during moderate exercise, and that these changes may be responsible for the secondary rise in ventilation.

On stopping exercise, there was usually a sharp decrease in the ventilation rate, as noted by Asmussen and Nielsen (26). The magnitude of this decrease varied greatly. The mean fall was 8.8 ± 2.6 l./min. Because of individual variation, there

TABLE IV

*Ventilation and arterial blood changes during early exercise and while breathing $CO_2$-enriched air at rest*

| Subject | Sampling time after starting exercise | Comment | Ventilation | Serum $CO_2$ | pH at 37° C. | $CO_2$ tension |
|---|---|---|---|---|---|---|
| | | | *(l./min., 25° C.)* | *(vol. %)* | | *(mm. Hg)* |
| R. M. | Rest | | 8.8 | 53.6 | 7.37 | 41 |
| | 32″–41″ | | 12.7* | 55.5 | 7.34 | 45 |
| | | Secondary vent. rise to 19.8 l./min. begins at 63 sec. | | | | |
| | 71″–82″ | | 19.8 | 55.8 | 7.28 | 52 |
| | 3′23″–3′33″ | | 36.5 | 50.9 | 7.21 | 55 |
| | Rest | 28 min. after exercise | 8.1 | 49.0 | 7.32 | 42 |
| | Rest | Breathing 3–4% $CO_2$ for 4 min. | 15.4 | 53.7 | 7.29 | 49 |
| E. S. | Rest | | 8.5 | 56.0 | 7.38 | 43 |
| | 16″–26″ | | | 55.5 | 7.34 | 46 |
| | | | 20.7* | | | |
| | 45″–55″ | | | 56.6 | 7.32 | 49 |
| | | Secondary vent. rise to 32.5 l./min. begins at 52 sec. | | | | |
| | 2′49″–3′04″ | | 43.6 | 53.1 | 7.27 | 51 |
| | Rest | 22 min. after exercise | 9.7 | 51.5 | 7.34 | 43 |
| | Rest | Breathing 4–5% $CO_2$ for 2½ min. | 25.2 | 56.5 | 7.28 | 53 |

* Average after first four to five respirations (about 15 seconds) until secondary rise in ventilation. Treadmill exercise at 4 mph and a 4% grade, subjects in poor training. A sharp "secondary rise" in ventilation is associated with an increase in $CO_2$ tension. Subjects show normal response to breathing $CO_2$ at rest.

was no significant difference between the magnitude of the immediate increase at the beginning of exercise and the decrease at the end.

### *The effect of breathing 5% $CO_2$*

In comparing the effect on ventilation of breathing 5% $CO_2$ at rest and during exercise, the difficulty arose that a number of our subjects were unable to continue exercise with the gas mixture long enough to reach a steady state. Accordingly, the mixture was given until it was evident from the respiratory tracing that the ventilation was no longer changing rapidly, and arterial samples were then taken. For comparison, the same procedure was adopted in the resting state. The average time on $CO_2$ at rest was two minutes and during exercise one and one-half minutes. The average time needed to reach a steady state on 5% $CO_2$ at rest probably exceeds five minutes and in individual cases may be much more (22, 29, 30). However, the breathing time used in the present study covers the period of rapid change in ventilation. In measuring the effect of $CO_2$ inhalation during exercise, it was necessary to select control levels for ventilation and arterial $CO_2$ tension. This was complicated by the fact that the subjects were not in a steady state, and ventilation was consequently increasing with time, apart from the effect of $CO_2$. As shown in Table I, during the same exercise period control measurements were made while the subject was breathing air, both before and after the administration of $CO_2$. The control value for ventilation while the subject was breathing $CO_2$ was estimated by assuming that the ventilation time curve would have followed a straight line between these two points if the subject had continued on air. The mean values for the two points on air, before and after giving $CO_2$, were 46.6 l./min. at three and one-half minutes of exercise, and 58.3 l./min. after seven and one-half minutes of total exercising time.[4] The mean estimated control ventilation during $CO_2$ breathing was 53.3 l./min., while the mean actual ventilation was 73.0 l./min. The estimated control ventilation is a little too low because the ventilation-time curve is not a straight line but is convex upward. The apparent effect of $CO_2$ is thereby magnified, but the effect is not great. In the second control period, measurements were made at an average of one and one-half minutes after changing from $CO_2$ to air. It might have been expected that measurements during this period would still be much influenced by the preceding $CO_2$ inhalation. However, the mean ventilation at this point (58.3 ± 4.4 l./min.) was not significantly different from that of the last control period in the oxygen experiments (56.0 ± 5.4 l./min.), which are described in the next section. In most of the latter, the preceding gas mixture was tank oxygen, which depresses ventilation instead of elevating it. It is concluded that ventilation in the second control period was not significantly influenced by the preceding gas mixture in either case. The mean $CO_2$ tension was approximately 47 mm. Hg in each control period. There was very little individual change between periods, and a simple average was taken for the $CO_2$ tension to be expected if the subject had continued on air. It appears that there is a rapid recovery from the effect of inhaling $CO_2$ during exercise.

TABLE V

*Mean effect on 10 subjects of breathing 5% $CO_2$ at rest and during exercise*

| State | Ventilation | $CO_2$ tension | Ventilation change/$CO_2$ tension change |
|---|---|---|---|
| | *(l./min., 25° C.)* | *(mm. Hg)* | *(l./min./mm. Hg)* |
| Rest | 8.3 ± 0.9 | 41.8 ± 1.5 | |
| Rest, 5% $CO_2$ | 15.5 ± 1.4 | 52.6 ± 1.4 | .80 ± .24 |
| Exercise | 53.3 ± 4.2* | 46.9 ± 2.1* | |
| Exercise, 5% $CO_2$ | 73.0 ± 7.5 | 65.5 ± 2.0 | 1.05 ± .20 |

* Expected values, calculated from measurements during exercise before and after the administration of $CO_2$. See text.

The mean results for 10 subjects are presented in Table V. The mean ventilation response to an increase in arterial $CO_2$ tension during exercise exceeds that at rest, but the difference is not statistically significant ($0.3 < P < 0.4$). It was suggested by Nielsen (22) that the respiratory center becomes more sensitive to $CO_2$ during exercise, and that this change is an important factor in the regulation of breathing during exercise. Under the conditions of the present experiment, there is no evidence that exercise has sensitized the respiratory center to $CO_2$ in the sense that the ventilatory response to a given increment in $CO_2$ tension becomes significantly greater. The same conclusion was reached by Grodins (31) from an examination of Nielsen's data. The magnitude of the response to 5% $CO_2$ at rest is somewhat lower than usual, probably because of the short exposure time. The mean pH fall as a result of breathing $CO_2$ was slightly greater during exercise than at rest. As a corollary to the first conclusion, there appears to be no evidence that exercise produces any marked respiratory sensitivity to a fall in arterial pH.

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[4] The four minutes between these sampling times included one and one-half minutes each on $CO_2$ and air, with approximately 30 additional seconds after each period which were occupied in completing the blood sampling and observing the ventilation rate to insure that it was not changing rapidly.

### *The effect of high and low oxygen concentrations*

In 1920 Briggs (24) observed that breathing oxygen-enriched air during severe exercise decreased the ventilation rate. The effect was greater the more severe the exercise, and for a given exercise rate the effect varied inversely with physical fitness. The finding has been confirmed abundantly (20, 22, 27, 32, 33). Conversely, it is known that ventilation for a given work rate is greater at high altitudes (13, 34, 35) or while breathing low-oxygen mixtures (15, 27, 36, 37) than it is at normal oxygen tensions. In the present study a trial was made of the effect on ventilation of changing from air to 15% $O_2$ in $N_2$ and to tank oxygen.

The first control measurements were made after about three minutes of exercise. The subject was then switched to 15% oxygen, and thereafter to air or tank oxygen. In all cases there were two or three control periods on air, as indicated in Table II. The time on each mixture was roughly one and one-half minutes before measurements were made. *The subjects did not know the order in which* mixtures were given and the changes were made without their knowledge. The method of determining "expected" ventilation and per cent oxygen *saturation from the control periods was the* same as described in the preceding section. The mean results for 11 subjects are presented in Table VI. Fifteen % $O_2$ caused a significant increase in ventilation ($P < .05$) and tank oxygen a significant decrease ($P < .01$), with reference to the rate on air. On switching gas mixtures, the change in ventilation rate was extremely rapid. This was particularly true in passing to oxygen, when a marked slowing was often evident within 10 seconds, or four to five breaths. With the ventilation changes there were corresponding changes in arterial $CO_2$ tension and pH. That is, with the lower ventilation under tank oxygen the $CO_2$ rose and the pH fell, while reverse changes occurred with 15% $O_2$. These secondary changes must have limited the extent of the oxygen effect, since they would force ventilation in the opposite direction.

TABLE VI

*Mean effect on 11 subjects of breathing 15% $O_2$ and tank $O_2$ during exercise*

| State | Ventilation | % Arterial $O_2$ saturation | Ventilation change |
|---|---|---|---|
| | (*l./min., 25° C.*) | | (*l./min.*) |
| Exercise, air | 52.1±4.8* | 96.9* | |
| Exercise, 15% $O_2$ | 61.6±6.5 | 85.7 | 9.5±3.1† |
| Exercise, air | 55.8±5.3* | 96.1* | |
| Exercise, tank $O_2$ | 47.4±3.9 | 100.0 | 8.4±2.3‡ |

* Expected values, calculated from measurements during control periods. See text.

† $p < .02$.

‡ $p < .01$.

It is of some interest to note that the mean change of $CO_2$ tension was only about 3 mm. Hg on changing from air to either gas mixture, which was less than might have been anticipated from the change in ventilation. The short exposure time may not have permitted the development of a full change in $CO_2$ tension. In addition, it is possible that the different gas mixtures may have influenced the rate of bicarbonate breakdown and consequently of $CO_2$ excretion. From the rate of total blood $CO_2$ loss, it is apparent that lactic acid was being formed rapidly. The rate of production of lactic acid during work can be markedly slowed by high oxygen mixtures or accelerated by low oxygen (20, 36, 38, 39).

*The effect on ventilation of changing arterial* oxygen tension is far greater during exercise of this severity than at rest. The effect on ventilation of inhaling high and low oxygen concentrations at rest has been studied by Dripps and Comroe (40). They found a small, immediate drop of 3.1% in ventilation on passing from air to oxygen. On 12% oxygen, which produced a mean fall of 17% in arterial saturation, approximately corresponding to that of the present study, there was an average ventilation increase of 0.5 l./min.

### *The effect of changes in arterial pH*

There has been some disagreement as to the role played by arterial pH in the regulation of breath-

TABLE VII

*Arterial blood pH, ventilation, and $CO_2$ tension in the post-exercise period*

| Subject | State | Ventilation | Arterial blood pH | Arterial blood $CO_2$ tension |
|---|---|---|---|---|
| | | (l./min., 25° C.) | | (mm. Hg) |
| R. M.* | Rest | 8.8 | 7.37 | 41 |
| | post-exercise 28 min. | 8.1 | 7.32 | 42 |
| E. S.* | Rest | 8.5 | 7.38 | 43 |
| | post-exercise 22 min. | 9.7 | 7.34 | 43 |
| S. | Rest | 7.1 | 7.37 | 44 |
| | post-exercise 6 min. | 8.4 | 7.33 | 45 |
| P. S. | Rest | 7.8 | 7.35 | 45 |
| | post-exercise 18 min. | 10.7 | 7.29 | 47 |

* Data repeated from Table IV.

ing during exercise. Barr (14, 41), Dill, Talbott and Edwards (8), and Nielsen (22) found little correlation between pH and ventilation. From studies on diabetic acidosis Kety and associates (42) have suggested that there is a threshold for respiratory response at about pH 7.2. Between this point and pH 7.0, ventilation increased as the hydrogen ion concentration rose. These investigators pointed out that the arterial $CO_2$ tension of their patients was simultaneously decreased. As emphasized by Gray (43) and Grodins (31) such a change could mask a stimulant effect which might otherwise appear at higher pH levels.

Some data on this point are available from the present study and are presented in Table VII. In the post-exercise period, ventilation and arterial $CO_2$ tension in these subjects have returned substantially to their former values, while the pH is still .04 to .06 units below the control level. The pH changes are not large, but it seems possible to conclude that small pH changes, produced in this way, do not evoke much respiratory response. No such data are available, from this study, for large pH changes. As pointed out above, the finding that exercise does not generally increase respiratory sensitivity to $CO_2$ also implies that it does not cause a marked increase in sensitivity to a fall in arterial blood pH, since the pH was decreased considerably both at rest and exercise by $CO_2$ inhalation.

### *Individual variation*

Individual variation is conveniently examined by reference to Figure 1. There is a striking difference among subjects in response to various stimuli.

The most consistent response is the immediate increase in ventilation on beginning exercise (Figure 1B). Even so, this shows up to 100% variation among subjects, and its magnitude is not closely related to the final ventilation volume. In Figure 1C is presented the abrupt fall in ventila-

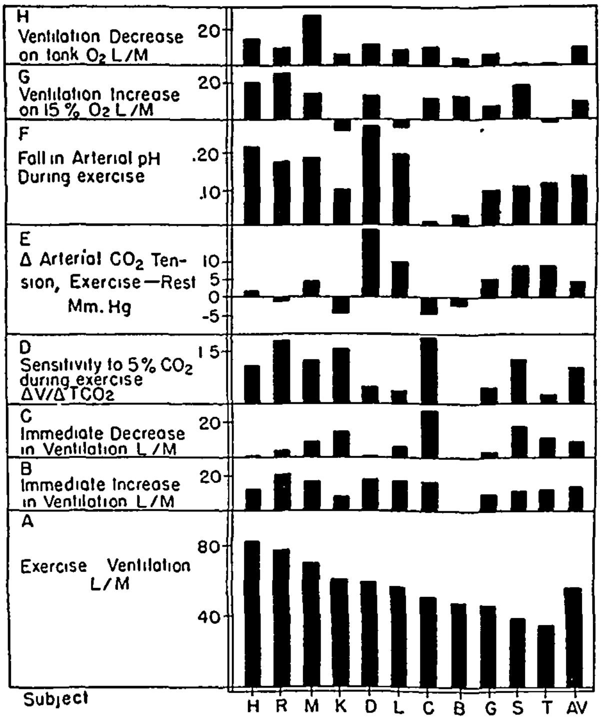

FIG. 1. VARIATION IN PERFORMANCE OF INDIVIDUAL SUBJECTS DURING EXERCISE

Columns at the extreme right represent the mean for each category. The values of categories A, B, C, E, and F are the averages of observations during two separate exercise periods, except for subject B, who had only one exercise period. A: ventilation during arterial blood sampling in the latter part of the exercise period. B: abrupt increase in ventilation on beginning exercise. C: abrupt decrease in ventilation on stopping exercise. D: increase in ventilation (l./min.) per mm. Hg rise in arterial $CO_2$ tension resulting from administration of 5% $CO_2$ during exercise. E: difference between arterial $CO_2$ tensions at exercise and rest. F: fall in arterial blood pH during exercise. G: increase in ventilation resulting from administration of 15% $O_2$ during exercise. H: decrease in ventilation resulting from administration of tank oxygen during exercise.

tion on stopping work. Here there is even a greater variation. In some subjects there is little or no immediate fall, while others show a fall greater than the original increase. The initial increase, because of its rapidity, has been considered neurogenic from the time of its description by Krogh and Lindhard, and the same etiologic reasoning is evidently applicable to the abrupt fall. Since the discovery by Harrison, Calhoun, and Harrison (44) of respiratory reflexes from passive limb motion, considerable attention has been devoted to this phase of respiratory control, most notably by Comroe and associates (45, 46), who have recently concluded that passive stretch reflexes can play only a minor part in respiratory regulation. Whether peripheral or central in origin, the present effect is of considerable magnitude. It is quite possible that the actual magnitude of this "neurogenic factor" during exercise is not fully measured by the abrupt changes in ventilation on starting and stopping exercise. A preliminary examination of this possibility has been made in subject H., who customarily shows an immediate increase in ventilation of about 12 l./min. on starting exercise and no immediate decrease on stopping, once a large ventilation volume has been reached. This subject was caused to exercise repeatedly for short periods with only brief recovery intervals. The immediate increase on passing from rest to exercise amounted in the first trial to 18 l./min., in the second, to 28 l./min., and in the third to 32 l./min., the last representing an immediate jump from 18 to 50 l./min. The possibility exists that the neurogenic factor may increase during exercise and become much larger than indicated by the average values for starting and stopping effects.

There is a very large range in sensitivity to arterial $CO_2$ tension, as shown by Figure 1D, representing ventilation increase per mm. of arterial $CO_2$ tension when 5% $CO_2$ is administered during exercise. Wide variation among subjects in response to carbon dioxide at rest has also been remarked (29, 30). Figure 1E presents the difference between arterial $CO_2$ tension during the latter part of exercise and the tension at rest. There is some correspondence between $CO_2$ sensitivity (Figure 1D) and the behavior of the arterial $CO_2$ tension during exercise. Where sensitivity is high, the $CO_2$ tension tends to fall or to rise only slightly during exercise. The large increase shown by D. is probably owing to anxiety and hyperventilation during the resting period, with a resultant subnormal $CO_2$ tension at rest. An exception is provided by S., who was sensitive to $CO_2$, but still showed a considerable increase in $CO_2$ tension during exercise. Evidently, the importance of $CO_2$ as a respiratory regulator during exercise is subject to considerable individual variation.

The reaction to 15% oxygen and tank oxygen is presented in Figures 1G and 1H. Once more, there was a considerable range of response. In several cases there was much difference in sensitivity to the two mixtures. The response to tank oxygen was a little more consistent than that to 15% oxygen.

## DISCUSSION

In 1944 Comroe (47) reviewed the evidence dealing with respiratory regulation during exercise and concluded that a number of factors must act together. Subsequent work, including the present, has confirmed the conclusion. Efforts have been made to quantitate the role played by various of these factors. Most of this work has been done with well-trained subjects. It is generally believed that neurogenic stimuli play some part, although there is disagreement as to whether reflexes originating from the limbs themselves are important or not (46, 48, 49). It has been held, on the one hand, that neurogenic stimuli are of primary importance in light to moderate exercise (50), and, on the other, that they are of little importance. Carbon dioxide has been invoked as a major factor on the ground that exercise increases the responsiveness of the respiratory center to this stimulus (22), and the significance of carbon dioxide as a major factor has been denied on the ground that the alveolar carbon dioxide tension remains unchanged or falls during exercise (47). pH change has not been considered of first-line importance by most observers (8, 22, 41), but its possible importance has recently been asserted (31). Arterial oxygen tension has been dismissed as a regulatory factor because changes in arterial oxygen during exercise are slight, and change in arterial oxygen tension over a considerable range yields little respiratory response at rest (20). A rise in body temperature during exercise may stimulate respiration, but this is slow to develop and probably would not be very effective in less than 10 minutes (51). Because it has often seemed difficult to account for the magnitude of ventilation during exercise, it has been recurrently postulated that an unidentified substance which acts as a respiratory stimulant is produced by working muscles.

In the present study, using untrained subjects, it is apparent that a number of stimuli were operative. Neurogenic factors, of whatever origin, played a significant part in initiating and maintaining ventilation. This stimulus may have been much larger than can be estimated from initial increases and terminal decreases in ventilation.

Arterial carbon dioxide tension appears to have been effective in increasing ventilation during exercise in at least some subjects. Since the respiratory center did not become highly sensitive to carbon dioxide during exercise, the effect as calculated after several minutes of exercise is not particularly large except perhaps in subject S., who was sensitive to carbon dioxide and had a substantial increase in arterial tension during exercise. In those subjects who have a rapid, early decrease in blood $CO_2$ content, presumably owing to lactic acid accumulation, a rising arterial $CO_2$ tension early in exercise may assist in increasing ventilation. As pointed out above, this may account for the "secondary rise" in ventilation early in exercise. In trained subjects this secondary rise has not been described, and trained subjects show little, if any, early increase in alveolar $CO_2$ tension (8, 22) corresponding to that found in the arterial blood of some of our untrained subjects.

The effect of changing the arterial oxygen tension is of considerable interest. The ventilation response is very rapid, and its magnitude is many times greater than at rest. This effect appears in exercise which is severe for the subject, and the effect becomes more pronounced as the severity increases (20, 24). The response is too immediate to make it likely to result from changes in blood concentration of some substance produced by anaerobically working muscles. It has been suggested by Asmussen and Nielsen (20) that an increase in oxygen tension could act by decreasing chemoreceptor sensitivity to such a substance. If so, one would need to postulate that the substance continues to be produced on oxygen at the same rate as on air, although lactic acid production is decreased by oxygen. This is because the results of these investigators show an immediate return of ventilation to the same steady rate as before when the subject is switched back to air. It seems a little simpler to assume that the chemoreceptor system becomes more sensitive to the arterial oxygen tension itself, or that the respiratory center becomes more sensitive to the "tone" which is known to exist at normal oxygen tensions in the resting state (40). In the latter case, the oxygen effect could be regarded as a variety of neurogenic stimulus which becomes more active the more severe the exercise. There is some evidence in animals for a respiratory drive from increased chemoreceptor tone, or increased responsiveness to the existing tone, during electrically induced work (49, 52). The development of this effect during exercise can make a significant contribution to the total ventilation. Normally, it can not provide for a very close regulation of ventilation because the effect does not vary greatly with a small change in oxygen tension. It is probably important in the close regulation of breathing during exercise at high altitudes and in patients with pulmonary disease who have a decreased arterial oxygen tension. For reasons outlined by Bohr (53) and Barcroft (54) and put to experimental trial by Lilienthal, Houston, and others (15, 35, 55), the arterial oxygen tension can fall markedly during exercise when it is already low at rest.

The large falls in arterial pH which occurred during exercise in some of our subjects would be expected to have some stimulant effect on respiration. Our experience, however, is similar to that of others in finding no close correlation between ventilation and pH in a given subject. As pointed out before, we find no evidence that the respiratory response to a fall in pH becomes very great during exercise.

Among the factors known to be operative in the present study, then, are a "neurogenic factor" which may increase during exercise, a changing arterial $CO_2$ tension, the development of what is provisionally regarded as an increased chemoreceptor tone to oxygen, and a considerable fall in pH. Recently, Gray (43) and Grodins (31) have approached the problem of multiple respiratory stimuli by assigning quantitative values to the stimulant effects of $CO_2$ tension, hydrogen ion concentration, and oxygen tension. If these values are accepted and the quantities measured, it is possible to draw conclusions as to the intensity and mode of action of additional stimuli which are required to account for the ventilation observed in a given situation. One of the most striking findings of the present study is the tremendous variability among untrained subjects in the response to different stimuli, as well as in the extent to which various stimuli develop during exercise. This suggests that any respiratory formula be applied only with reservations to a single subject or even to small groups of subjects, particularly when they are not in good physical training.

This great variability among normal subjects in poor physical condition is in contrast with the much more uniform performance of subjects in good training. It is probable that still greater deviations will occur in the course of disease. Present concepts of the relative importance of various respiratory stimuli in muscular exertion have been gained largely through the study of well-conditioned subjects. It does not appear likely that these concepts can be carried over intact to persons with acute or chronic disease.

## SUMMARY

1. Observations have been made on ventilation and arterial blood changes in 11 untrained subjects during short periods of moderately severe treadmill exercise. Particular attention was paid to the ventilation changes at the start and conclusion of work, to the course of ventilation in early exercise, to the effect on ventilation of breathing gas mixtures having, respectively, an increased $CO_2$ content, a low oxygen, and a high oxygen content, and to associated changes in arterial $CO_2$ tension, pH and per cent oxygen saturation.

2. With untrained subjects there may be large changes during exercise in arterial $CO_2$ content, $CO_2$ tension, and pH, and these may begin to develop very early in exercise.

3. Exercise did not increase significantly the sensitivity of the respiratory center to arterial $CO_2$ tension, as measured by increase in ventilation per mm. Hg increase in $CO_2$ tension.

4. An attempt is made to estimate in semi-quantitative terms the role played by various respiratory stimuli in regulating ventilation during exercise. These stimuli are a "neurogenic" effect; an oxygen effect, which is provisionally regarded as an increase in sensitivity of the chemoreceptor system to oxygen tension; fall in blood pH; and arterial $CO_2$ tension. There is great variation among untrained subjects in the relative importance of these factors.

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# THE EFFECT OF NOR-EPINEPHRINE UPON PULMONARY ARTERIOLAR RESISTANCE IN MAN[1]

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(Submitted for publication January 6, 1951; accepted, March 12, 1951)

Nor-epinephrine is a sympathomimetic drug similar in structure to epinephrine, but lacking a methyl group on the nitrogen atom contained in the latter compound (1). Its levo-isomer is twice as active as the optically inactive preparation. Some investigators believe that l-nor-epinephrine is identical with sympathin E (1); others feel that it is more likely that nor-epinephrine is the precursor of epinephrine at adrenergic nerve endings (2, 3). Goldenberg and associates (1) found that in man l-nor-epinephrine increases total peripheral resistance, and in further contrast to epinephrine, does not increase the cardiac output. Goldenberg also (1) found a consistent elevation in pulmonary artery pressure during the infusion of l-nor-epinephrine in man. However, since he did not measure pulmonary venous pressure, one cannot determine from his study the primary site of action of l-nor-epinephrine in producing transient pulmonary hypertension. For these reasons it seemed desirable to investigate further the action of l-nor-epinephrine upon the pulmonary circulation.

The purpose of the study reported here is to determine how l-nor-epinephrine produces pulmonary arterial hypertension in man. In man the pulmonary venous pressure may not ordinarily be measured directly. However, Hellems and coworkers (4) have described a method of obtaining pulmonary "capillary" pressure, and have shown that in animals the pulmonary "capillary" pressure varies with the pulmonary venous pressure. If pulmonary artery pressure, pulmonary "capillary" pressure and cardiac output are determined simultaneously, the pulmonary arteriolar resistance may be calculated (5). If nor-epinephrine produces pulmonary hypertension by pulmonary arterial constriction, then a rise in pulmonary arteriolar resistance is to be expected during the pulmonary hypertension resulting from its exhibition.

## MATERIAL

Thirteen subjects were studied. Eleven had normal hearts as shown by physical examination, X-ray, and the electrocardiogram. One (E. B.) was convalescing from beriberi heart disease, with only residual tachycardia. One (C. F.) had a systolic murmur at the cardiac apex and a pulmonary "capillary" pressure tracing suggestive of incompetency of the mitral valve (6). C. F. had also moderate elevation of the mean pulmonary arterial pressure while in the resting state. As controls, six subjects with various types of cardio-pulmonary disease were studied.

## METHOD

All subjects except E. B. were studied in the fasting condition, sedated by 0.1 or 0.2 gm. of seconal given two to two and a half hours prior to study. Cardiac catheterization was performed as described previously (7). A double lumen catheter was used, so that pulmonary "capillary" pressure and pulmonary arterial pressure could be measured simultaneously. Pulmonary "capillary" pressure was obtained by the method of Hellems and co-workers (4). The criteria of satisfactory pulmonary "capillary" pressures were (*1*) the nature of the pressure curve, showing "a" and "c" waves; (*2*) the peripheral location of the catheter tip in the lung; and (*3*) the securing of a specimen of blood saturated with oxygen from the wedged catheter tip. This last was not always possible, since in some instances, no blood could be obtained from the wedged catheter tip. Brachial artery pressure was obtained through an 18 gauge indwelling Cournand needle. Simultaneous electrocardiograms, ballistocardiograms, brachial arterial, pulmonary arterial, and pulmonary "capillary" pressures were recorded by means of a five channel optical oscillograph.

Cardiac outputs were determined by the direct Fick method as described previously (8). Duplicate samples of expired air were analyzed for $CO_2$ and $O_2$ and required to check within 0.03%. Duplicate blood samples, collected during the collection of expired air, were required to check within 0.2 vol.%.

Resting cardiac outputs were obtained after the catheter had been in place 15 to 30 minutes or more. After the

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[1] This study was supported by Research Contract V1001 M-432 Veterans Administration.

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TABLE I

*The effect of l-nor-epinephrine upon pulmonary artery and brachial artery pressure*

| Subject | Before nor-epinephrine | | During nor-epinephrine | | 10 to 20 minutes after nor-epinephrine | |
|---|---|---|---|---|---|---|
| | Mean PA pressure | Mean BA pressure | Mean PA pressure | Mean BA pressure | Mean PA pressure | Mean BA pressure |
| | *mm. Hg* | *mm. Hg* | *mm. Hg* | *mm. Hg* | *mm. Hg* | *mm. Hg* |
| 1. H. H. 39 W.M. Paresis | 15 | 75 | 19 | 103 | 16 | 87 |
| 2. S. M. 40 C.M. Convalescent pneumonia | 15 | 113 | 19 | 150 | 16 | 146 |
| 3. H. P. 48 W.M. CNS syphilis | 16 | 130 | 17 | 146 | 14 | 125 |
| 4. G. Y. 46 W.M. Convalescent pneumonia | 18 | 92 | 22 | 101 | 18 | 104 |
| 5. V. H. 27 W.M. Epilepsy | 10 | 114 | 13 | 118 | 8 | 116 |
| 6. C. H. 49 C.F. Paresis | 19 | 111 | 27 | 124 | 19 | 115 |
| 7. L. A. 43 W.M. Neuritis | 13 | 113 | 18 | 127 | 13 | 112 |
| 8. E. J. 43 C.M. Leukemia | 16 | 100 | 27 | 124 | — | — |
| 9. C. F. 50 C.M. Mitral incompetency | 23 | 101 | 31 | 126 | 30 | 104 |
| 10. E. B. 28 W.F. Convalescent beriberi | 17 | 110 | 24 | 133 | — | 114 |
| 11. J. D. 42 C.M. Bronchiectasis (*minimal*) | — | 100 | — | 113 | — | — |
| 12. W. S. 26 C.M. Convalescent pneumonia | — | 92 | — | 124 | — | 90 |

blood and gas samples for the resting cardiac output had been collected, the subjects were given 0.2 to 0.4 microgram of l-nor-epinephrine[3] per kilogram body weight per minute into the distal aperture of the catheter lying within the pulmonary artery. When a maximum effect upon the blood pressures had been obtained, usually within three to five minutes, blood and gas samples for a second cardiac output were collected. The infusion of l-nor-epinephrine was continued during the collection of samples. Pressure recordings were obtained immediately before and immediately after the collection of blood samples. In six cases a third cardiac output and set of pressure recordings were obtained 10 to 20 minutes after discontinuing the l-nor-epinephrine infusion. More than one resting cardiac output would have been desirable but was not obtained because of the danger of fatiguing the subject.

Six control subjects were studied by the above method. They received fluids comparable in volume and rate of infusion to those received by the subjects to whom nor-epinephrine was administered. Following resting measurements of cardiac output and pressures, 5% dextrose solution was infused into the pulmonary artery for a period of time roughly equivalent to that required for the infusion of l-nor-epinephrine. Then resting measurements of cardiac output and pressures were repeated.

Mean pulmonary arterial, brachial arterial and pulmonary "capillary" pressures were determined by planimetry. Measurements were made over two or more respiratory cycles. Pressures taken just before and just after each cardiac output were averaged in obtaining the final result. A point 10 cm. anterior from the back of the horizontal subject was taken as the zero point in pressure measurements.

Pulmonary arteriolar resistance was calculated by the formula:

$$R = \frac{PA - PC}{CO} \times 1{,}332.$$

Where R = arteriolar resistance in dynes/sec./cm.$^{-5}$
PA = mean pulmonary artery pressure
PC = mean pulmonary "capillary" pressure
CO = cardiac output in cc./sec.

Total peripheral resistance (TPR) was determined by the formula

$$TPR = \frac{BA}{CO} \times 1{,}332.$$

Where TPR = total peripheral resistance in dynes/sec./cm.$^{-5}$
BA = mean brachial artery pressure
CO = cardiac output in cc./sec.

Systemic peripheral resistance was determined according to the formula:

$$SPR = \frac{BA - RA}{CO} \times 1{,}332.$$

Where SPR = systemic peripheral resistance in dynes/sec./cm.$^{-5}$
BA = mean brachial arterial pressure
CO = cardiac output in cc./sec.
RA = mean right auricular pressure

## RESULTS

*Pulmonary arterial and brachial arterial pressure* (*Table I*). In each of 10 subjects a definite rise in pulmonary artery pressure occurred. The mean rise in pulmonary artery pressure was 5 mm. Hg

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[3] Supplied through the courtesy of Winthrop-Stearns Company, Buffalo, New York.

TABLE II

*Statistical table of changes induced by l-nor-epinephrine*

| | No. cases | t—1% level | t—5% level | Observed t | p value * |
|---|---|---|---|---|---|
| 1. Pulmonary artery mean pressure | 10 | 3.250 | — | 5.7 | <0.001 |
| 2. Brachial artery mean pressure | 12 | 3.106 | — | 6.99 | <0.001 |
| 3. Pulmonary "capillary" mean pressure | 9 | 3.355 | — | 6.33 | <0.001 |
| 4. Cardiac output | 10 | — | 2.262 | 2.270 | <0.05 |
| 5. Heart rate | 12 | 3.106 | — | 4.3 | <0.01 |
| 6. Total peripheral resistance | 11 | — | 2.228 | 2.6 | <0.05 |
| 7. Arteriovenous oxygen difference | 11 | — | 2.228 | 1.85 | <0.1 |
| 8. Stroke volume | 11 | — | 2.228 | 1.94 | <0.1 |
| 9. Correlation of rise in P. A. and P. C. mean pressure | 9 | r = 0.76† | — | r = 0.85† | <0.01 |

* From Fisher and Yates (10).
† Correlation coefficient (11).

or 33% over the resting value. Each of 12 subjects had a definite rise in brachial arterial pressure. The mean rise in brachial artery pressure was 20 mm. Hg or 19%. Ten to 20 minutes after cessation of nor-epinephrine administration, the pressure had fallen in all cases in the pulmonary artery, and in all cases save one in the brachial artery. However, 10 to 20 minutes was insufficient time for complete subsidence of nor-epinephrine effect in every case. The changes in both pulmonary artery and brachial artery pressures were statistically significant (9) at the $p < 0.001$ level (Table II).

*Cardiac output* (*Table III*). A significant rise (*i.e.,* 10% above the resting level [8]) in cardiac output was found in only one of the 11 cases. In this case the cardiac output rose still further after withdrawal of the nor-epinephrine; therefore the observed elevation was attributed to anxiety rather than the effect of the drug. A significant fall in output was observed in five subjects. In the five remaining subjects there was no significant change in cardiac output. Statistical analysis (Table II) revealed a significant decline in cardiac output when the group was considered as a whole ($p = .05$). The low cardiac index of subject C. F. is attributed to heavy sedation rather than to cardiac failure, since the subject had a normal arteriovenous oxygen difference of 3.9 vol.%. This subject was apparently unduly sensitive to barbiturates, and was roused with difficulty during the study, although he had received only 0.2 gm. of seconal. His oxygen consumption was quite low, being only 74.6 cc./sq. m./min.

*Pulmonary "capillary" pressure and pulmonary arteriolar resistance* (*Table IV*). There was a definite rise in pulmonary "capillary" pressure during the administration of nor-epinephrine in each instance. The mean rise was 6 mm. Hg or 64%, close to the mean rise in pulmonary artery pressure of 5 mm. Hg. Ten to 20 minutes after cessation of the nor-epinephrine infusion the pulmonary "capillary" pressures had fallen toward the original levels in all cases. The pulmonary arteriolar resistance showed no significant change in four subjects, a definite fall in three, and a definite rise in two subjects. The pulmonary arteriolar resistances showed much less tendency then pulmonary artery

TABLE III

*The effect of l-nor-epinephrine upon cardiac output and heart rate*

| Subject | Before nor-epinephrine | | During nor-epinephrine | | After nor-epinephrine | |
|---|---|---|---|---|---|---|
| | Cardiac Index | Heart rate | Cardiac Index | Heart rate | Cardiac Index | Heart rate |
| | *L/sq. m./min.* | *per min.* | *L/sq. m./min.* | *per min.* | *L/sq. m./min.* | *per min.* |
| 1. H. H. 39 W.M. | 3.0 | 72 | 2.9 | 60 | 3.8 | 76 |
| 2. S. M. 40 C.M. | 3.1 | 66 | 3.2 | 50 | 3.3 | 63 |
| 3. H. P. 48 W.M. | 3.0 | 64 | 2.6 | 50 | — | 68 |
| 4. G. Y. 46 W.M. | 2.3 | 82 | 3.3 | 65 | 3.8 | 88 |
| 5. V. H. 27 W.M. | 3.2 | 65 | 3.3 | 55 | 3.9 | 64 |
| 6. C. H. 49 C.F. | 3.4 | 82 | 3.4 | 78 | — | 86 |
| 7. L. A. 43 W.M. | 4.3 | 92 | 3.5 | 60 | 4.7 | 88 |
| 8. E. J. 43 C.M. | 3.3 | 65 | 3.4 | 70 | — | 80 |
| 9. C. F. 50 C.M.* | 1.9 | 45 | 1.3 | 38 | 1.9 | 50 |
| 10. J. D. 42 C.M. | 2.7 | 72 | 2.5 | 68 | 3.0† | 68† |
| 11. W. S. 26 C.M. | 3.6 | 70 | 2.6 | 55 | — | 74 |
| 12. E. B. 28 W.F. | — | 98 | — | 68 | — | — |

* Heavily sedated.
† Second control before drug administration.

TABLE IV

*The effect of l-nor-epinephrine upon pulmonary "capillary" pressure and pulmonary arteriolar resistance*

| Subject | Before nor-epinephrine | | During nor-epinephrine | | After nor-epinephrine | |
|---|---|---|---|---|---|---|
| | P. C. | Pulm. res. | P. C. | Pulm. res. | P. C. | Pulm. res. |
| | *mm. Hg* | *dynes/sec./cm.$^{-5}$* | *mm. Hg* | *dynes/sec./cm.$^{-5}$* | *mm. Hg* | *dynes/sec./cm.$^{-5}$* |
| 1. H. H. 39 W.M. | 10 | 89 | 17 | 40 | 12 | 46 |
| 2. S. M. 40 C.M. | 9 | 95 | 13 | 95 | 10 | 90 |
| 3. H. P. 48 W.M. | 7 | 133 | 9 | 138 | 5 | — |
| 4. G. Y. 46 W.M. | 11 | 131 | 17 | 65 | 12 | 69 |
| 5. V. H. 27 W.M. | 6 | 66 | 11 | 37 | 7 | 27 |
| 6. C. H. 49 G.F. | 9 | 151 | 15 | 163 | 6 | — |
| 7. L. A. 43 W.M. | 9 | 41 | 12 | 75 | 6 | 63 |
| 8. E. J. 43 C.M. | 7 | 114 | 17 | 122 | — | — |
| 9. C. F. 50 C.M. | 14 | 202 | 22 | 326 | 16 | 333 |

mean pressures to return toward the original level in those cases where they were altered. The observed elevation in pulmonary "capillary" pressure was significant statistically ($p < 0.001$) (Table II).

*Total peripheral resistance and heart rate (Tables III and V).* A significant increase in total peripheral resistance (TPR) was found in nine of 11 subjects during the infusion of nor-epinephrine. There was no significant change in one subject, and a decline in resistance in another. The mean rise was 497 dynes/sec./cm.$^{-5}$ or 31%. The heart rate slowed in 11 of the 12 subjects. The one subject who did not show slowing (E. J.) demonstrated a further rise in heart rate after the cessation of nor-epinephrine, suggesting a masking of the drug effect by some extraneous factor. Both heart rate and peripheral resistance returned toward resting values 10 to 20 minutes after withdrawal of the l-nor-epinephrine. The observed bradycardia was statistically significant at the $p < 0.001$ level and the observed increase in total peripheral resistance was significant statistically at the $p < 0.05$ level (Table II).

TABLE V

*The effect of l-nor-epinephrine upon total peripheral resistance*

| Subject | Before nor-epinephrine | During nor-epinephrine | After nor-epinephrine |
|---|---|---|---|
| | TPR | TPR | TPR |
| | *dynes/sec./cm.$^{-5}$* | *dynes/sec./cm.$^{-5}$* | *dynes/sec./cm.$^{-5}$* |
| 1. H. H. 39 W.M. | 1,223 | 1,698 | 1,119 |
| 2. S. M. 40 C.M. | 1,727 | 2,222 | 2,160 |
| 3. H. P. 48 W.M. | 1,918 | 2,474 | — |
| 4. G. Y. 46 W.M. | 1,704 | 1,312 | 1,188 |
| 5. V. H. 27 W.M. | 1,787 | 1,802 | 1,478 |
| 6. C. H. 49 C.F. | 1,539 | 1,708 | — |
| 7. L. A. 43 W.M. | 1,142 | 1,609 | 1,045 |
| 8. E. J. 43 C.M. | 1,227 | 1,502 | — |
| 9. C. F. 50 C.M. | 2,523 | 4,557 | 2,637 |
| 10. J. D. 42 C.M. | 1,615 | 1,976 | 1,405* |
| 11. W. S. 26 C.M. | 1,170 | 2,187 | — |

* Duplicate observations before drug administration.

*Right atrial pressure, right ventricular end diastolic pressure, and peripheral venous pressure.* In each of three subjects in whom observations were made pressures within the right side of the heart rose during administration of the drug. In subject W. S. right atrial pressure was 29 mm. water in the resting state. During l-nor-epinephrine administration, the pressure rose to 82 mm. water. After the drug was discontinued, right auricular pressure fell to 38 mm. water. In subject E. B., right ventricular end diastolic pressure rose from 5 mm. Hg to 14 mm. Hg during l-nor-epinephrine infusion. Right atrial pressure also rose during the drug infusion but unfortunately the pressure was not measured at its peak and the rise was less striking than that in the right ventricular end diastolic pressure. In subject F. H. there was a striking rise in right atrial pressure during l-nor-epinephrine infusion (Figures 1 and 2). A peripheral venous pressure curve was obtained simultaneously with the right atrial and peripheral artery pressure curve in this subject (Figure 2). There was an approximately equal rise in pressure in the peripheral vein and right atrium approximating 10 mm. Hg in the right atrium and 11 mm. Hg in the peripheral vein. However, the peak pressure rise occurred earlier in the peripheral vein than in the right atrium (Figure 1).

MEAN PRESSURE CHANGES IN RIGHT ATRIUM, FEMORAL ARTERY AND PERIPHERAL VEIN IN PATIENT F.H. DURING L-NOR-EPINEPHRINE INFUSION

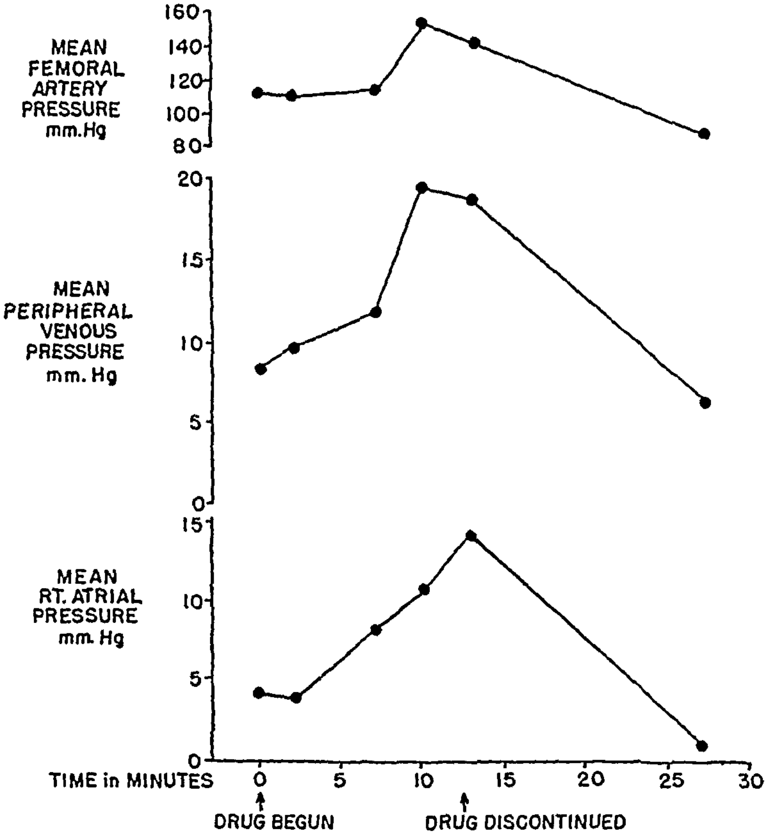

FIG. 1.

TABLE VI

*Control subjects*

| | First resting study | | | | | | Second resting study | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | BA mean | PA mean | PC mean | CO *L/min.* | TPR | Pulm. res. | BA mean | PA mean | PC mean | CO *L/min.* | TPR | Pulm. res. |
| 1. R. J. 69 C.M. hypertension | — | 16.8 | 6.8 | 3.6 | — | 223 | — | 16.4 | 8.8 | 3.4 | — | 181 |
| 2. M. L. 52 W.F. emphysema | 87.3 | 20.8 | 5.8 | 3.6 | 1,961 | 337 | 90.3 | 21.5 | 5.1 | 3.5 | 2,092 | 380 |
| 3. M. S. 49 W.M. mitral stenosis | — | 53.8 | — | 3.7 | — | — | — | 49.5 | — | 3.9 | — | — |
| 4. J. D. 48 C.M. bronchiectasis | 99.6 | — | 12.8 | 4.9 | 1,615 | — | 93.4 | — | 11.8 | 5.3 | 1,405 | — |
| 5. D. H. 61 W.M. cor pulmonale | 65.3 | 38.4 | — | 4.4 | 1,963 | — | 65.7 | 36.3 | — | 4.2 | 2,050 | — |
| 6. F. B. 18 C.F. sickle cell anemia | — | 18.6 | — | 8.6 | — | — | — | 16.8 | — | 7.9 | — | — |

Mean pressures are expressed in mm. Hg.
Resistances are expressed in dynes/sec./$cm^{-5}$.
BA = brachial artery.
PA = pulmonary artery.
PC = pulmonary "capillary."
CO = cardiac output.

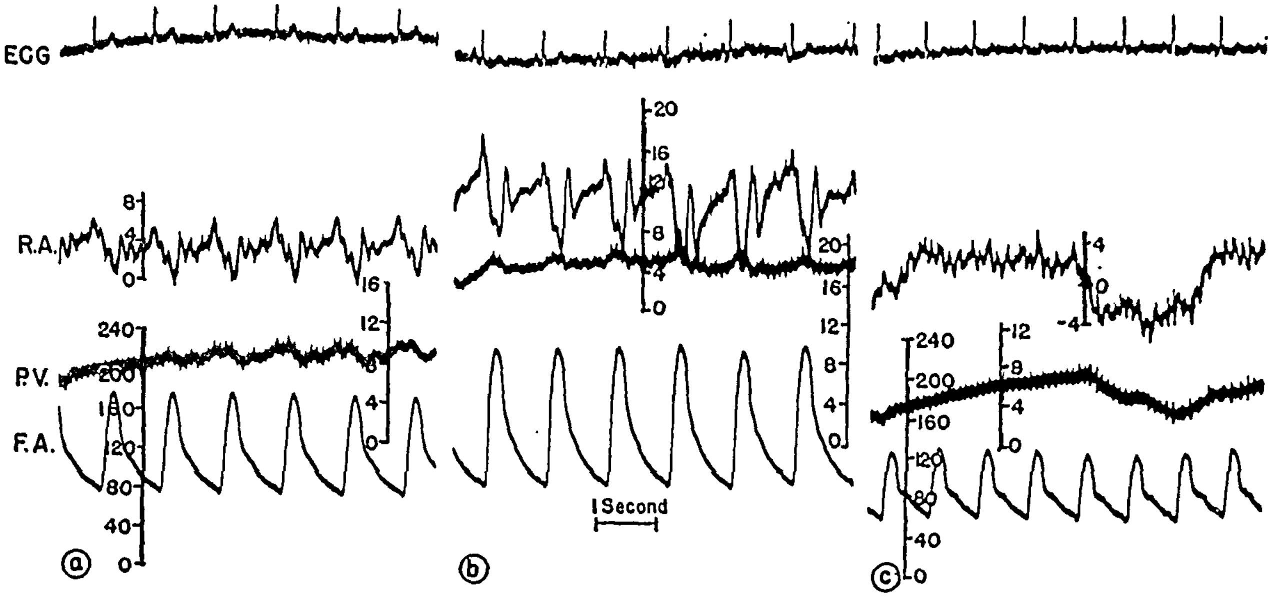

Fig. 2. Excerpted from Pressure Tracing of Patient F. H.

Section (a) is before l-nor-epinephrine; (b) is at the peak response 13 minutes after beginning the infusion of l-nor-epinephrine; (c) shows the return of pressure to resting levels 14 minutes after cessation of l-nor-epinephrine infusion. In (a), mean femoral artery pressure is 111.6 mm. Hg; mean peripheral vein pressure is 8.4 mm. Hg; mean right atrial pressure is 4.2 mm. Hg. In (b), the mean femoral artery pressure has risen to 141.2 mm. Hg; mean peripheral vein pressure, to 18.7 mm. Hg; mean right atrial pressure, to 14.2 mm. Hg. In (c) mean femoral artery pressure has fallen to 88.4 mm. Hg; mean peripheral vein pressure, to 6.3 mm. Hg; mean right atrial pressure, to 1.1 mm. Hg.

In order to exclude changes in right atrial pressure as a cause of increased total peripheral resistance, the observed values for right atrial pressure should be subtracted from the brachial pressure in the formula. This could not be done because right atrial pressures were not routinely measured. In one subject (W. S.) in whom this could be done, the systemic peripheral resistance was 1143 dynes/sec./cm.$^{-5}$ before and 2082 dynes/sec./cm.$^{-5}$ during l-nor-epinephrine, showing an increase of 82%.

In the six control subjects (Table VI), the second resting cardiac output varied less than 10% from the resting measurement. The mean variation in pulmonary artery pressure was 1.7 mm. Hg. The observed variations in brachial artery pressure, pulmonary "capillary" pressure, total peripheral resistance, and pulmonary arteriolar resistance are seen in Table VI.

## DISCUSSION

The hemodynamic changes observed during an infusion of l-nor-epinephrine consisted of increased pulmonary artery and brachial artery pressures, increased peripheral resistance, diminished cardiac output, and bradycardia, and confirm the observations of Goldenberg (1). The bradycardia was also observed by Judson and co-workers (12).

The mechanism of the increase in pressure in the pulmonary and peripheral arteries is not the same. There was a statistically significant elevation in total peripheral resistance during l-nor-epinephrine administration; on the other hand, in only two of the nine cases studied was there an increase in pulmonary arteriolar resistance. In one of these (C. F.) the pulmonary "capillary" pressure rose within the critical range of 20–25 mm. Hg, at which level pulmonary resistance increases as a protective mechanism to prevent pulmonary edema (5). Thus it is quite probable that the rise in resistance in case C. F. was not a direct effect of l-nor-epinephrine, but an effect secondary to the increase in pulmonary venous pressure. An increase in pulmonary "capillary" pressure was observed in all cases following l-nor-epinephrine, and the mean increase was nearly the same as the mean increase in pulmonary artery pressure. Therefore, the usual mechanism whereby l-nor-epinephrine produces pulmonary hypertension in man is through an increase in pulmonary "capillary" or venous pressure.

The rise in pulmonary artery pressure following l-nor-epinephrine infusion is due to a rise in left atrial pressure for the following reasons. First, there is no consistent rise in pulmonary arteriolar resistance after the drug, thereby excluding constriction of the pulmonary arterioles as the mechanism of pressure elevation. Second, there is a significant correlation between the rise in the pulmonary artery and pulmonary "capillary" mean pressures during l-nor-epinephrine infusion (Table II). Third, there is a definite rise in pulmonary "capillary" pressure following l-nor-epinephrine which implies a rise in pulmonary venous pressure and thus a rise in left atrial pressure. Fourth, there is a rise in right atrial pressure after the drug, which also implies a concomitant rise in left atrial pressure (13).

One may speculate further as to the mechanism of elevation of right atrial pressure following l-nor-epinephrine. Ranges and Bradley (14) and Bradley and Parker (15) in attempting to explain the rise in right atrial pressure after paredrinol and angiotonin, considered heart failure unlikely because there was no evidence of cardiac dilatation. They believed that an increase in venous tone, decreasing the total volume of the vascular system, might be responsible. Iglauer and Altschule (16) have shown that other sympathomimetic drugs—adrenaline and paredrine—increase the peripheral venous pressure. Ranges and Bradley also mentioned an increase in "cardiac tone" as the cause of the elevated right atrial pressure following angiotonin.

TABLE VII

*Effect of l-nor-epinephrine upon A-V oxygen difference and upon stroke volume of the heart*

| Subject | Stroke volume (*cc.*) | | A-V difference (*cc./100 cc. blood*) | |
|---|---|---|---|---|
| | Before l-nor-epinephrine | During l-nor-epinephrine | Before l-nor-epinephrine | During l-nor-epinephrine |
| 1. H. H. | 68 | 81 | 3.7 | 4.5 |
| 2. C. F. | 71 | 58 | 3.9 | 5.5 |
| 3. S. M. | 79 | 108 | 4.7 | 5.7 |
| 4. H. P. | 85 | 94 | 4.5 | 5.2 |
| 5. G. Y. | 53 | 95 | 4.7 | 4.7 |
| 6. V. H. | 78 | 96 | 3.2 | 3.6 |
| 7. C. H. | 70 | 74 | 4.0 | 3.2 |
| 8. L. A. | 86 | 105 | 2.7 | 3.5 |
| 9. E. J. | 100 | 94 | 5.3 | 4.4 |
| 10. J. D. | 69 | 67 | 4.0 | 4.1 |
| 11. W. S. | 89 | 82 | 3.0 | 4.6 |

Although there was an increase in the mean A–V oxygen difference of 12% in the subjects studied, there was also an increase in the mean stroke volume of 13% (Table VII). The changes in stroke volume and A–V oxygen difference were not significant statistically (Table II). Therefore it is believed that the decrease in cardiac output during l-nor-epinephrine infusion does not represent congestive heart failure (1). The striking increase in peripheral venous pressure observed in Subject F. H., reaching its peak earlier than the peak rise in right atrial pressure during the l-nor-epinephrine infusion, suggests that increased peripheral venous tone may indeed be responsible for the rise in intra-atrial pressure by decreasing the capacity of the vascular system. The change here is quite unlike that in congestive heart failure, where the gradient between pressure in peripheral veins and right atrium is diminished rather than increased (17), the pressure in the two sites often becoming almost equal as the pressure rises.

One must also consider the possibility that peripheral venous constriction is the mechanism primarily responsible for the hypertension induced by l-nor-epinephrine in the systemic circuit. Since a rise of 20 mm. Hg, the observed mean elevation in mean peripheral arterial pressure, was much greater than any of the measured increases in mean right auricular pressure (this relationship holding true in the three cases where both pressures were determined) and since the cardiac output is decreased or unchanged after l-nor-epinephrine, one is justified in stating that there is definite increase in systemic peripheral resistance during administration of the drug. In one case where systemic peripheral resistance was calculated, there was a striking increase of 82% during l-nor-epinephrine. Thus one may conclude that arterial or capillary constriction contributes to the observed increase in total peripheral resistance despite the rise in right atrial pressure.

## SUMMARY AND CONCLUSIONS

The hemodynamic response of 13 subjects to the infusion of l-nor-epinephrine was studied. During l-nor-epinephrine infusion, the following changes occurred: a statistically significant mean rise in pulmonary artery pressure; a significant rise in mean brachial artery pressure; a decrease in the cardiac output in five subjects, remaining unchanged in five. There was a statistically significant mean rise in pulmonary "capillary" pressure. There was a statistically significant increase in total peripheral resistance. There was no significant increase in pulmonary arteriolar resistance. There was a definite and marked rise in right atrial pressure in each of three subjects.

It is concluded that the pulmonary hypertension seen during the administration of l-nor-epinephrine is not due to constriction of the pulmonary arteries, but rather to an increase in pulmonary venous pressure; it is also concluded that l-nor-epinephrine produces constriction of peripheral arteries or capillaries as well as peripheral veins.

## ACKNOWLEDGMENT

The authors gratefully acknowledge the technical assistance of Mrs. Mary Damron and Mr. Harold Perlman.

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# BLOOD LEVELS OF ADENINE NUCLEOTIDES[1]

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(Submitted for publication December 23, 1950; accepted, March 17, 1951)

## INTRODUCTION

The metabolic significance of the adenine nucleotides (1, 2) and the reports of the therapeutic action of muscle adenylic acid and adenosine triphosphate in various pathological states (3–12) has stimulated interest in the possible mechanism of action in these compounds.

Recently a rapid reliable method was developed by one of us (13) for measuring total adenine, adenylic acid (AMP), adenosine diphosphate (ADP) and adenosine triphosphate (ATP). We have applied this method to the study of blood since it is the only human tissue which can be conveniently studied.

Before studies could be undertaken on pathologic bloods, we felt that normal bloods should be examined for total nucleotide content as well as for possible content of adenylic acid, adenosine diphosphate and adenosine triphosphate. That adenine nucleotides are present in blood has been known since 1914. The first completely adequate quantitative data are those of Buell in 1935 (14) who estimated total nucleotide nephelometrically after precipitating with uranium and then silver, from a neutralized trichloracetic acid filtrate. Apart from describing the procedure itself, Buell reported that the nucleotides are present principally in the red cells (indeed the value varies with the hematocrit); that the level for any one individual over a period of several months is fairly constant, and that the average value for males is about 11.5 mgm.% and for females 10.0 mgm.% (expressed as adenine).

Our interest, as indicated above, was not only in the total nucleotide, but also in the constitution of the nucleotide. We also felt that the Buell procedure for estimating nucleotides was much too cumbersome. We, therefore, concentrated our attention on the preparation of filtrates which could be assayed directly.

The procedure used in our work is a spectrophotometric assay on perchloric acid filtrates, using in modified form the enzyme techniques of Kalckar (15–17).

In addition to presenting observations on the concentration of the various nucleotides on a group of four normal subjects (three men and one woman) over a period of about eight months, data will be presented on the effects of intramuscular injections of muscle adenylic acid, adenosine triphosphate and yeast adenylic acid on the levels of the above compounds.

## ANALYTICAL PROCEDURE

### a) *Estimation of total adenine*

Total adenine nucleotide was assayed by drawing blood, initially under oxalate, and immediately deproteinizing 1 ml. with 4 volumes of cold 2% perchloric acid. The protein precipitate was washed with an additional volume of cold 2% perchloric acid. The combined supernatant solutions collected after centrifugation were adjusted to pH 7.0 and made to a final volume of 10 ml. One ml. of filtrate was added to a tube containing 3.5 ml. $H_2O$ and 0.5 ml. M/10 phosphate buffer at pH 7.4. The resulting solution when read in the spectrophotometer shows an absorption maximum at 258 mμ, corresponding to the absorption maximum of adenine nucleotides in perchloric acid. Total nucleotide was calculated as adenine using muscle adenylic acid as a standard.

### b) *Estimation of AMP, ADP and ATP*

Adenylic acid, adenosine diphosphate and adenosine triphosphate were assayed in the same filtrates according to the procedure described by Albaum and Lipshitz (13), in the following way: One ml. aliquots of the filtrate were placed in three separate tubes. Into the first tube 0.5 ml. 0.1 M malonate buffer pH 5.9, 0.03 ml. of adenylic acid deaminase (containing 15 μgm. protein N) and water to make a final volume of 5.0 ml. were pipetted and the change in density in the spectrophotometer at 265 mμ recorded at the end of 10 minutes. The second tube received buffer, water, 0.1 ml. $MgCl_2$ (10 mgm./ml.), 0.01 ml. myokinase (containing 4 μgm. protein N) and deaminase and again the change in density at 265 mμ was recorded at the end of 10 minutes. To the third tube was added buffer, water, $MgCl_2$, myokinase, 0.1 ml. 0.5 M glucose, 0.01 ml. hexokinase (containing 7 μgm. protein N) and the deaminase and once more the change in density determined. Any

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[1] This work was supported by a grant to Brooklyn College from the Ernst Bischoff Company, Ivoryton, Conn.

change in tube 2 over that occurring in tube 1 is due to the presence of ADP. Since only 1 mole of AMP is formed from 2 moles of ADP, the difference between tubes 1 and 2 is multiplied by a factor of 2. The third tube records the change in density due to AMP, ADP and ATP. Since the concentration of AMP and ADP are now known (by reading the adenine due to these compounds from a standard curve derived from the change in density of known concentrations of these nucleotides) and the total adenine is known from tube 3, the difference between the AMP and ADP adenine and the total adenine gives the value for the ATP adenine.

The reactions discussed above are represented by the following equations:

$$\text{(1) AMP} \xrightarrow{\text{adenylic acid deaminase}} \text{Inosinic Acid}$$

$$\text{(2) 2ADP} \xrightarrow{\text{myokinase}} \text{ATP} + \text{AMP}$$

$$\text{(3) ATP} + \text{glucose} \xrightarrow{\text{hexokinase}} \text{ADP} + \text{glucose-6-phosphate}$$

Before experiments were undertaken on the distribution of the compounds, several preliminary runs were carried out to see whether the method as described was reliable, *i.e.*, could added nucleotide be recovered and measured? The results of one such experiment are shown in Table I. The total nucleotide accountable for as AMP, ADP and ATP in this sample of blood was 6.4 μgm. It is apparent that added ATP can be recovered indicating that the method is reliable.

Early in our studies on the concentration of the various nucleotide fractions in oxalated human blood, it was found that ATP content decreased significantly within the first hour, though not in the first five minutes. For this reason, in the early work which was done on oxalated blood, the samples were pipetted into perchloric acid within one minute of withdrawal. To avoid the inconvenience of this procedure, the stability of the nucleotide in heparinized blood was investigated. Table II shows the percentage of total purine found in the ATP, AMP and ADP fractions, respectively, in freshly drawn blood, and in the same blood samples after 90 minutes. The experiment was run at room temperature and at 37° C. in the presence of a mixture of potassium oxalate and ammonium oxalate, of which the oxalate concentration was 112 mgm. %, and in the presence of 5 mgm. % of heparin. It is clear that the ATP is very significantly split to ADP and AMP in oxalated blood, while it is quite stable in the heparinized blood.

TABLE I

*Recovery of ATP when added to perchloric acid filtrate of blood*

1. Total AMP, ADP, and ATP found in blood sample = 6.4 μgm.
2. ATP added to blood sample = 8.2 μgm.
3. Total AMP, ADP, and ATP in blood and added ATP
   calculated (1 + 2) = 14.6 μgm.
   found = 14.3 μgm.

TABLE II

*Effect of standing on adenine nucleotides in blood*

| Temperature | | Time | % of total purine as | | |
|---|---|---|---|---|---|
| | | | ATP | AMP | ADP |
| | | min. | | | |
| Room | Oxalated blood | 0 | 68 | 0 | 0 |
| | | 90 | 15 | 28 | 25 |
| | Heparinized blood | 0 | 58 | 0 | 0 |
| | | 90 | 59 | 0 | 4 |
| 37° C. | Oxalated blood | 0 | 40 | 0 | 5 |
| | | 90 | 12 | 20 | 7 |
| | Heparinized blood | 0 | 39 | 0 | 5 |
| | | 90 | 44 | 0 | 0 |

Adenylic acid deaminase (Schmidt's deaminase) was prepared according to the method of Kalckar (15); myokinase, according to Colowick and Kalckar (18); hexokinase was prepared in partially pure form according to the method of Berger, Slein, Colowick and Cori (19) through step No. 3 (fractionation with ethanol at 0° C.) of the isolation procedure. The enzymes were kept in a deep freeze and were found to be stable over long periods of time.

Muscle adenylic acid used for injection was supplied by the Ernst Bischoff Co., Ivoryton, Conn. as My-B-Den (1 ml. ampules containing 20 mgm. as the sodium salt). In some experiments ampules containing 100 mgm./ml. were used. ATP was also obtained from the Ernst Bischoff Co. and was administered as a solution of the sodium salt.

## EXPERIMENTAL

### *Total nucleotide content of blood*

The total nucleotide content of the blood of the four subjects used in the experiments expressed as mgm. % adenine are shown in Figure 1 (closed circles). Several conclusions may be drawn from these data: *1.*) While there is some variability in total nucleotide, the levels remain fairly uniform over a period of about eight months. The variability is no greater than one finds in the levels of other blood constituents under normal conditions. *2.*) The levels obtained are similar to those obtained by Buell using the nephelometric procedure, so that we appear to be measuring the same thing that Buell measured.

### *Nucleotide present as AMP, ADP and ATP*

That portion of the total nucleotide accountable for as AMP, ADP and ATP is also shown in Figure 1 (open circles). It is immediately clear that the level of these compounds shows considerably more variation than does the total nucleotide.

They may vary anywhere between 30 and 90% of the total nucleotide. Some of the early samples were collected before breakfast, the later ones after breakfast; and there appeared to be no consistent difference.

To further illustrate the difference in variability between the values for the total purine and those for the known nucleotide (AMP, ADP and ATP) we have calculated the mean, standard deviation and coefficient of variability for a large number of individual determinations (n = 78). For the total purine the following values were obtained: m = 10.5 mgm.%, the standard deviation 0.84, the coefficient of variability 8.0%. The corresponding values for the known nucleotide were m = 6.0 mgm.%, the standard deviation 1.58, and the coefficient of variability 26.5%.

*Effect of injection of adenylic acid*

The fact that the amount of nucleotide present as AMP, ADP and ATP is variable from day to day (see Figure 1), and that the total nucleotide remains fairly uniform, suggests that the former are individually in some kind of loose equilibrium with the total nucleotide. Can this equilibrium be shifted, *e.g.*, by the injection of adenylic acid? In these experiments 20 mgm. ampules of muscle adenylic acid as the sodium salt (My-B-Den) were used. Blood was taken from the antecubital vein, and immediately thereafter the contents of an adenylic acid ampule were injected into the other arm intramuscularly. Additional blood samples were withdrawn from the first arm 30, 60 and 90 minutes after the injection. The results of three typical runs are shown in Table III. It should be noted that most of the blood samples contained ADP and ATP, but no adenylic acid initially. After injection in all cases the ADP decreased and the ATP increased. The ATP level in two of the three cases began to fall at the end of 60 minutes and adenylic acid appeared. The total purine accountable for as AMP, ADP and ATP approached the value for the total nucleotide. In many cases the increases obtained in the known AMP, ADP and ATP fractions were not as marked as those shown, but they almost always occurred. In one case no increase was obtained in the first run, though in a subsequent run it did occur.[2]

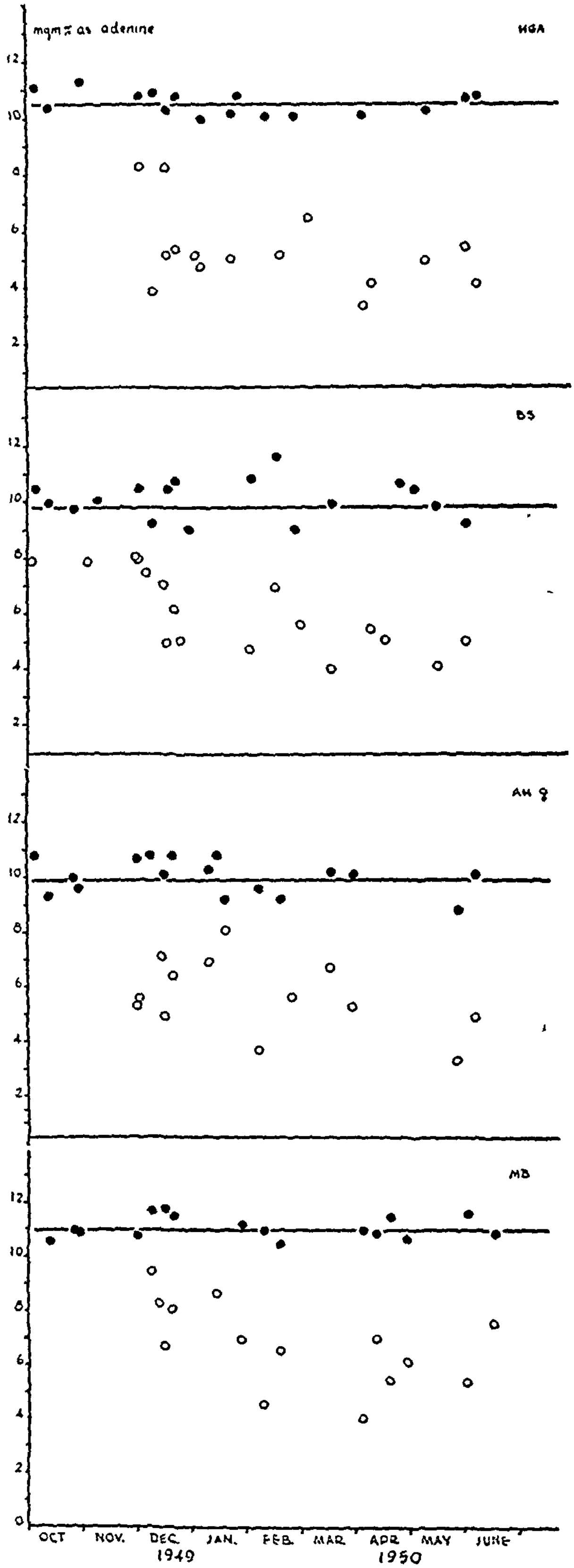

FIG. 1. TOTAL PURINE (CLOSED CIRCLES) AND NUCLEOTIDES PRESENT AS AMP, ADP AND ATP (OPEN CIRCLES) FOR A GROUP OF FOUR SUBJECTS (THREE MEN AND ONE WOMAN) OVER A PERIOD OF EIGHT MONTHS

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[2] Since this paper went to press, the response of five additional normal subjects to the injection of muscle adenylic acid was tested. Four of the five showed a response similar to that in Table III. One subject showed no change in blood nucleotide. A later run on the same subject also gave a negative response.

TABLE III

*Effect of intramuscular injection of 5-adenylic acid: 20 mgm. (My-B-Den) on blood nucleotides (AMP, ADP, ATP)*

| Time (*minutes*) | AMP | ADP | ATP | Total |
|---|---|---|---|---|
| AH 1/18/50—Total purine 9.2 mgm. % | | | | |
| 0 | 0.0 | 6.5 | 1.7 | 8.2 |
| →30 | 0.0 | 4.5 | 4.3 | 8.3 |
| 60 | 0.0 | 0.8 | 8.0 | 8.8 |
| 90 | 1.2 | 0.0 | 8.0 | 9.2 |
| HA 1/20/50—Total purine 10.2 mgm. % | | | | |
| 0 | 0.0 | 4.1 | 1.0 | 5.1 |
| →30 | 0.0 | 3.4 | 4.4 | 7.8 |
| 60 | 0.0 | 0.0 | 10.0 | 10.0 |
| 90 | 2.4 | 0.0 | 7.1 | 9.5 |
| MB 1/23/50—Total purine 11.2 mgm. % | | | | |
| 0 | 0.0 | 3.7 | 3.3 | 7.0 |
| →30 | 0.0 | 3.4 | 6.8 | 10.2 |
| 60 | 0.0 | 2.5 | 7.6 | 10.1 |
| 90 | 1.1 | 3.4 | 4.0 | 8.5 |
| BS 3/13/50—Total purine 10.0 mgm. % | | | | |
| 0 | 0.0 | 0.0 | 4.1 | 4.1 |
| →30 | 0.0 | 0.0 | 7.7 | 7.7 |
| 60 | 0.0 | 0.0 | 9.1 | 9.1 |
| 90 | 0.0 | 0.0 | 7.7 | 7.7 |

The magnitude of the increases obtained was entirely out of proportion to the amount of adenylic acid injected. The increase in concentration of nucleotide expected if all of the injected nucleotide were simultaneously to appear in the blood would not exceed 0.1 to 0.2 mgm.%. The effect, therefore, appears to be an indirect one of some kind. This is further indicated by the fact that when a 100 mgm. dose of muscle adenylic acid was injected (see Table IV), the increase obtained was no greater than that obtained with the 20 mgm. dose. With the 100 mgm. dose there was a transitory increase in total purine which was not ob-

TABLE IV

*Effect of intramuscular injection of 5-adenylic acid: 100 mgm. (My-B-Den) on blood nucleotides*

| Time (*minutes*) | AMP | ADP | ATP | Total |
|---|---|---|---|---|
| HA 3/1/50 Initial total purine 10.1 mgm.—30 minute total purine 11.2 mgm. % | | | | |
| 0 | 0.0 | 0.9 | 5.7 | 6.6 |
| →30 | 0.2 | 0.0 | 9.0 | 9.2 |
| 60 | 0.3 | 0.0 | 7.2 | 7.5 |
| 90 | 0.0 | 0.9 | 5.9 | 6.8 |
| MB 2/31/50 Initial total purine 10.2 mgm. %—30 minute total purine 12.0 mgm. % | | | | |
| 0 | 0.5 | 0.0 | 6.1 | 6.6 |
| →30 | 0.0 | 0.0 | 9.6 | 9.6 |
| 60 | 0.3 | 0.5 | 8.3 | 9.1 |
| 90 | 0.3 | 4.6 | 4.3 | 9.2 |

TABLE V

*Effect of intramuscular injection of 3-adenylic acid (yeast): 20 mgm. on blood nucleotides*

| Time (*minutes*) | AMP | ADP | ATP | Total |
|---|---|---|---|---|
| RS Total purine—12.1 mgm. % | | | | |
| 0 | 0 | 0 | 9.0 | 9.0 |
| →30 | 0 | 0 | 9.0 | 9.0 |
| 60 | 0 | 0 | 9.3 | 9.3 |
| 90 | 0 | 0 | 9.3 | 9.3 |
| LIC Total purine—8.2 mgm. % | | | | |
| 0 | 0 | 0 | 4.2 | 4.2 |
| →30 | 0 | 0 | 4.5 | 4.5 |
| 60 | 0 | 0 | 4.8 | 4.8 |
| 90 | 0 | 0 | 4.8 | 4.8 |

TABLE VI

*Blood cell levels of adenine nucleotides after addition of 5-adenylic acid (10 mgm. %) to whole blood* in vitro

| Time (*minutes*) | | AMP | ADP | ATP (*mgm. %*) |
|---|---|---|---|---|
| 0 | Control | 0.0 | 0.0 | 5.9 |
| | Experimental | 0.0 | 0.0 | 5.8 |
| 1 | Control | 0.0 | 0.0 | 5.5 |
| | Experimental 10 mgm. % AMP | 0.0 | 0.0 | 5.3 |
| 3 | Control | 0.0 | 0.0 | 5.2 |
| | Experimental | 0.0 | 0.0 | 5.3 |
| 15 | Control | 0.0 | 0.0 | 5.8 |
| | Experimental | 0.0 | 0.0 | 5.8 |

TABLE VII

*Effect of intramuscular injection of ATP (equivalent to 20 mgm. AMP)*

| Time (*minutes*) | AMP | ADP | ATP | Total (*mgm. %*) |
|---|---|---|---|---|
| AH Total purine—10.1 mgm. % | | | | |
| 0 | 0.0 | 0.0 | 5.3 | 5.3 |
| →30 | 0.0 | 0.0 | 7.0 | 7.0 |
| 60 | 0.0 | 0.0 | 7.9 | 7.9 |
| 90 | 0.0 | 0.0 | 7.2 | 7.2 |

TABLE VIII

In vitro *destruction of ATP by whole blood—ATP added, 5.2 mgm. %*

| Time (*minutes*) | AMP | ADP | ATP |
|---|---|---|---|
| 0 | 0.7 | 0.0 | 4.5 |
| 15 | 1.3 | 0.6 | 3.3 |
| 30 | 1.3 | 0.9 | 3.0 |
| 60 | 1.8 | 0.6 | 2.8 |
| 90 | 3.0 | 0.0 | 2.2 |

served with the 20 mgm. dose. Within 60 minutes the level had returned to normal.

The possibility that the observed effects of muscle adenylic acid on blood nucleotide levels was due to psychogenic or other adventitious factors, was excluded by the results of experiments carried out under identical conditions, but substituting yeast adenylic acid for muscle adenylic acid (Table V).

That the effect of the injected adenylic acid was an indirect one was demonstrated by adding AMP directly to whole blood. The experiment was carried out in the following way. Blood was removed under heparin from the antecubital vein of one of the subjects. Ten ml. of this blood were placed in each of two tubes. One ml. from each tube was removed and centrifuged; the plasma was poured off and the cells deproteinized as before. The filtrates were assayed for AMP, ADP and ATP. To one of the blood samples was then added sufficient AMP to produce an increase of nucleotide of 10.0 mgm.%. At the end of one, three and 15 minutes, 1 ml. samples were removed, centrifuged and deproteinized as before and once more AMP, ADP and ATP were determined.

The results are shown in Table VI. It is apparent that not only does added AMP produce no effect on the distribution of other nucleotides in the cell, but it does not even appear to penetrate the red blood cell. Experiments carried out over a longer time interval gave similar results.

The injection of ATP in a dose containing the same adenine content as a My-B-Den ampule yields results which are similar to that obtained with the latter compound (Table VII). That the effect in this case may be due to the adenylic acid in ATP, rather than ATP itself is suggested by the observation that ATP placed in contact with blood is rapidly split (Table VIII).

## DISCUSSION

In spite of the importance of the adenine nucleotides in so many critical life processes, this is the first report which presents data on the relative concentration of the different nucleotides in human blood. It is now possible to compare these values with corresponding values in different pathological states. Such experiments are already in progress.

From a medical standpoint it would be of great interest to know whether the reported clinical therapeutic effects of adenylic acid as well as earlier reports on ATP, are mediated through effects of this type. It would be especially interesting to know whether changes similar to those in the blood cells occur in other cells of the body (myocardium, central nervous system, nerves, glands, etc.), and whether, perhaps, the as yet unexplained actions of substances other than adenylic acid may operate through similar mechanisms. Comparison of the behavior of the adenine nucleotides in normal individuals with similar processes in various disease states may explain some of the puzzling phenomena in clinical medicine, and may possibly provide a mechanism for some of the "diseases of unknown etiology."

One point bears special emphasis: the lability of the equilibrium of the total nucleotide. We have shown that under certain conditions we can shift the balance in favor of ATP. Very recently Leder, Perlzweig, and Handler (20) have shown that when nicotinamide is added to washed erythrocytes, an eight to ten fold increase occurs in a pyridine nucleotide which behaves like DPN in a fluorometric assay. Since DPN contains adenylic acid, the latter must be derived from the same labile reservoir which under our conditions is converted to ATP. This indicates that the nucleotide may be "shifted" to compounds other than ATP under appropriate conditions.

## SUMMARY

1. A technique is described for measuring the total adenine nucleotide as well as the adenylic acid, adenosine diphosphate, and adenosine triphosphate content of blood. The total nucleotide remains fairly uniform over long periods of time, while the known nucleotide (AMP, ADP and

ATP) shows considerable variation from week to week.

2. In freshly drawn blood the level of known nucleotide is maintained for at least 90 minutes when the blood is drawn under heparin; when drawn under oxalate, the ATP is rapidly converted to ADP and AMP.

3. The magnitude of the ATP level of blood is increased by the intramuscular injection of My-B-Den (muscle adenylic acid) and ATP, but not by yeast adenylic acid. The effect obtained appears to be an indirect one since larger doses produce no more of an increase than do lower doses. That the effect is indirect is further borne out by the observation that nucleotide (AMP) added directly to blood does not appear to enter the cells.

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# BUTANOL-EXTRACTABLE IODINE OF SERUM[1]

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(Submitted for publication January 11, 1951; accepted, March 19, 1951)

It has been demonstrated chemically that the iodine bound to proteins of the serum is predominantly composed of thyroxine (1–4). The concentration of this protein-bound or serum precipitable iodine (SPI) appears to be closely correlated with the activity of the thyroid gland (5–7). It is probably the most precise clinical criterion of such activity that has yet been extensively employed. It is, therefore, generally conceded that SPI ordinarily represents circulating thyroid hormone. In studies of medically treated hyperthyroid patients, however, a number of values for SPI proved unexpectedly high in comparison with other measures of thyroid activity (5, 7). Danowski and associates (8–10) reported that SPI of normal individuals and pregnant women rose after the administration of potassium iodide. It had also been discovered that certain iodine-containing compounds commonly used for diagnostic and therapeutic purposes formed combinations with the proteins of the serum and therefore were measured with SPI (11). For these reasons it seemed desirable to examine more closely the nature of SPI and to find a more specific measure of circulating thyroid hormone.

Taurog and Chaikoff (4) utilized *N*-butanol to extract thyroxine-like compounds from serum. This solvent, originally employed by Leland and Foster (12), can be freed from diiodotyrosine and inorganic iodine by means of an alkaline reagent suggested by Blau (13). Taurog and Chaikoff (4) found that 73 to 93 per cent of the iodine of normal plasma behaved like thyroxine inasmuch as it remained in butanol extracts after these had been washed with Blau's reagent. Danowski and associates (8–10) compared the iodine extracted by butanol with SPI in the sera of normal individuals, pregnant women, and persons taking potassium iodide. The mean value for iodine extraced by butanol in the sera from 23 healthy males and nonpregnant females and 21 pregnant women amounted to 69 per cent of SPI (10). In five normal persons the ratio, butanol-extractable iodine (BEI): SPI varied from 0.65 to 0.82 (8). BEI remained unchanged after the administration of potassium iodide, but both SPI and inorganic iodine increased (8, 9).

By a simplification of the procedure of Taurog and Chaikoff (4), BEI of a series of subjects of varied categories has been measured and compared with SPI.

## METHOD FOR DETERMINATION OF BUTANOL-EXTRACTABLE IODINE

### *Apparatus*

*Pyrex centrifuge tubes* with heavy glass walls containing 25 cc. below a ground glass stopper. Holes in both the stopper and tube serve as an outlet for compressed butanol vapor.

Apparatus for digestion, distillation and colorimeter recordings of micro SPI procedure (6).

### *Reagents*

*Sulfuric acid* 10 per cent by volume.

N-*Butanol,* analytical reagent.

*Sodium hydroxide* 4 N., to each liter of which are added 50 gm. of anhydrous sodium carbonate (4, 13).

Reagents for digestion, distillation and colorimetry of micro SPI procedure (6).

### *Procedure*

To 1 cc. of serum, mixed with 0.2 cc. of 10 per cent $H_2SO_4$ in the special centrifuge tubes, are added rapidly, in a fine stream, 5 cc. of butanol during continuous shaking. The mixture is shaken without the stopper to prevent loss around the ground glass joint. The tube is centrifuged at 2,000 r.p.m. for exactly eight minutes to insure a firmly packed precipitate which can be completely dispersed in subsequent portions of butanol.

After centrifugation the butanol is siphoned into a second centrifuge tube through a glass tube of such short length and small diameter as to prevent loss of butanol. To the precipitate in the original tube are added 4 cc. of butanol. Before tightening the stopper, excess vapor in the tube escapes through the vent. After vigorous shaking the stopper is removed cautiously to prevent loss of butanol. When the mixture has been centrifuged for five

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[1] This investigation was supported (in part) by a research grant from the U. S. Public Health Service.

minutes the second portion of butanol is siphoned into the tube containing the original butanol extract. A third portion of 3 cc. of butanol is added to the precipitate. Shaking, centrifugation for 10 minutes and siphoning are repeated as for the second extract.

To the combined three portions of butanol is added an approximately equal volume of 4 *N.* sodium hydroxide containing 5 per cent sodium carbonate. After vigorous shaking the stopper is removed cautiously to prevent loss of butanol. The mixture is centrifuged at 2,000 r.p.m. for at least 12 minutes.

The lower layer of alkali is then removed by suction or siphoning through a tube of small diameter equipped with a stopcock which permits the tube to be inserted through the butanol layer for the removal of the alkali without admitting any butanol in the tube. Two additional extractions with the alkaline reagent are performed in a similar manner. Finally, a fourth portion of the reagent is added. After shaking, the layers are either centrifuged or allowed to separate overnight.

The butanol is drawn, with suction, into an iodine digestion flask (6). When all but a one-quarter inch layer of butanol has been removed, the ground glass stopper is held above the centrifuge tube and washed with 3 to 4 cc. of butanol. This final portion of butanol is then added to the flask containing the original extract. These final 3 to 4 cc. of butanol permit the removal of most of the thin butanol layer remaining after the first suction without admission of any of the alkali and also wash the original butanol extract from the inside of the siphon tube.

The butanol is then evaporated to dryness in a boiling water bath with suction applied through wide glass tubes inserted in the necks of the flasks. The dried material is freed from the inside of the flask by adding 5 to 8 cc. of double distilled water. Eight cc. of 2 *N.* potassium permanganate and 18 cc. of 27 *N.* sulfuric acid are added and determination of iodine is conducted as in the steps for digestion, distillation and measurement of SPI (6).

The reagent blank is determined by extracting a mixture of 12 cc. of butanol and 0.2 cc. of 10 per cent sulfuric acid with the alkaline reagent in the same manner used in treating the butanol extract of serum. The butanol is extracted four times. After evaporation of the butanol, the visible residue of alkali is loosened by adding 5 to 8 cc. of water before the addition of a definite quantity of potassium iodide or iodate. The necessity of adding a known quantity of potassium iodide for the determination of an accurate blank has been discussed (6). The blank with the reagents employed has varied from 0.4 to 1.3 gamma per cent of iodine. It appears to arise from both the butanol and the alkali.

### *Notes*

Considerable difficulty was encountered in extracting serum with butanol until a pH of 3.5 to 4.0 was obtained by adding sulfuric acid, using liquid brom phenol blue as an indicator. This pH is the same as that used by Taurog and Chaikoff (14, 15) and Rall (3) to extract thyroxine from an alkaline extract of thyroid gland. In the actual determination no brom phenol blue is used since it has been found that addition of 0.2 cc. of 10 per cent sulfuric acid gives the correct pH with 1 cc. portions of serum.

In Taurog and Chaikoff's (4) method the 45 cc. of butanol extracts of 3 cc. of plasma were washed with 50 cc. of alkaline reagent in separatory funnels. Decrease of the volume of 1 cc. of serum permits centrifugation and reduces the time required to layer the solutions. Preliminary separation of the organic from the inorganic iodine in serum by washing the precipitated proteins and using the washed precipitate is not necessary in this method. Values obtained by extracting untreated serum were the same as those obtained by extracting the washed zinc hydroxide precipitate of the same serum. For example, BEI's obtained from untreated serum were 7.2 and 7.3 gamma per cent, whereas BEI's obtained from the washed protein precipitate of the same serum were 7.8 and 6.9 gamma per cent (average 7.3 gamma per cent).

## RESULTS

*Recovery of thyroxine.* Thyroxine, diiodotyrosine and inorganic iodide were added to butanol or to sera. The quantities of thyroxine added were of the magnitude that might be expected in sera from normal subjects or patients. The mixtures were then analyzed for BEI by the method described above. The sera were also analyzed by the same method before the additions. The results of these experiments appear in Table I. Multiple measurements were made to ascertain the extent of analytical variation.

Of thyroxine added to butanol in aqueous solution (Experiments 1 to 4) 93 to 102 per cent was recovered. When thyroxine was added to sera (Experiments 5 to 8), the increment recovered amounted to 88 to 103 per cent of the thyroxine added. The addition of large amounts of diiodotyrosine (Experiments 4 and 10) or a massive quantity of inorganic iodine (Experiment 9) did not affect these recoveries. Both diiodotyrosine and inorganic iodine appeared to be quantitatively removed by the washing with alkali. It had been shown earlier (16) that a fraction of diiodotyrosine adheres to protein, which is not removed by the method of washing employed in the measurement of SPI.

Although recoveries have been expressed in terms of per cent, there is nothing in these experiments to indicate that any precise fraction of thyroxine is recovered or lost. The differences between theoretical and analytical values in no instance exceed analytical errors; nor are they pro-

TABLE I

*Recovery of thyroxine iodine by butanol extraction**

| Calculated iodine† | | | No. determinations | Determined BEI | | | Iodine recovered |
|---|---|---|---|---|---|---|---|
| Serum‡ | Thyroxine | Total | | Min. | Max. | Aver. | |
| γ % | γ % | γ % | | γ % | γ % | γ % | % |
| 0 | 5.3 | 5.3 | 3 | 5.0 | 5.1 | 5.1 | 96 |
| 0 | 8.5 | 8.5 | 7 | 7.3 | 8.3 | 7.9 | 93 |
| 0 | 10.6 | 10.6 | 2 | 10.2 | 10.6 | 10.4 | 98 |
| 0 | 10.4 | 10.4§ | 2 | 10.5 | 10.7 | 10.6 | 102 |
| 3.5 | 7.6 | 11.1 | 2 | 9.4 | 10.2 | 9.8 | 88 |
| 4.6 | 4.8 | 9.4 | 3 | 8.2 | 9.0 | 8.6 | 91 |
| 4.0 | 7.5 | 11.5 | 3 | 10.5 | 10.5 | 10.5 | 91 |
| 3.8 | 8.0 | 11.8 | 3 | 11.5 | 12.5 | 12.2 | 103 |
| 4.2 | 0 | 4.2‖ | 3 | 3.8 | 3.9 | 3.9 | 93 |
| 3.8 | 0 | 3.8§ | 3 | 2.9 | 4.0 | 3.6 | 95 |

* The thyroxine was given by Squibb Co. for investigational purposes. The iodine content by Squibb's analytical report was 64.4 per cent.

† 1 cc. samples were used for analyses.

‡ Calculated from BEI of original serum.

§ 32.0 gamma per cent diiodotyrosine iodine added.

‖ 22.4 gamma per cent diiodotyrosine iodine and 1,000 gamma per cent inorganic iodine added to serum with an SPI of 4.2 gamma per cent. Since the patient was receiving no iodine nor thio drug, the original serum was not subjected to butanol extraction.

portional to the amounts of iodine in the samples. It would be improper to conclude from these experiments that recovery was not complete. It may be that a small quantity, of the order of 0.4 gamma per cent, is lost in the course of the procedure.

*BEI and SPI of normal serum.* Duplicate or triplicate measurements of BEI and SPI in the sera of six normal individuals are presented in Table II. The difference, SPI − BEI, varied from 0 to 1.3 gamma per cent, averaging 0.6 gamma per cent. These differences again are of doubtful significance and not correlated with the amount of iodine present. The consistency with which SPI exceeds BEI in these and other experiments suggests, however, that the extractable iodine retained by butanol is actually slightly smaller than the SPI.

*BEI and SPI of untreated patients.* In Table III BEI and SPI of 17 patients with a variety of conditions are compared. None of the subjects had been given thyroid or antithyroid preparations, with the exception of Du, who had had no treatment for six months. There is reasonable doubt about B9489, who was extremely unreliable and given to self-medication. The first four were normal pregnant women. Cases 5 to 8 inclusive had large goiters. With the possible exception of B9489 all seemed to be in a euthyroid state. Cases 9 to 12 were suffering from obvious hyperthyroid-

TABLE II

*SPI and BEI in normal individuals*

| Subject | Sex | SPI | BEI | SPI−BEI |
|---|---|---|---|---|
| | | γ % | γ % | γ % |
| H | F | 6.2 | 4.9 | 1.3 |
| C | F | 4.1 | 4.1 | 0 |
| F | F | 5.3 | 4.0 | 1.3 |
| A | M | 6.7 | 6.4 | 0.3 |
| E | M | 5.0 | 4.8 | 0.2 |
| C | M | 4.5 | 4.0 | 0.5 |
| Average | | 5.3 | 4.7 | 0.6 |

TABLE III

*SPI and BEI of untreated patients*

| Subject | SPI | BEI | SPI−BEI |
|---|---|---|---|
| | γ % | γ % | γ % |
| C45201 | 7.3 | 5.7 | 1.6 |
| B53121 | 7.3 | 7.6 | −0.3 |
| | 8.4 | 7.0 | 1.4 |
| Du | 8.4 | 8.2 | 0.2 |
| Wa | 7.7 | 5.7 | 2.0 |
| C43177 | 5.0 | 4.0 | 1.0 |
| C7178 | 6.9 | 5.8 | 1.1 |
| C41885 | 7.0 | 6.0 | 1.0 |
| B9489 | 13.7 | 7.8 | 5.9 |
| Na | 10.5 | 9.2 | 1.3 |
| B86525 | 10.6 | 10.9 | −0.3 |
| A81700 | 11.5 | 10.8 | 0.7 |
| B7958 | 16.6 | 15.1 | 1.5 |
| Be | 4.1 | 4.3 | −0.2 |
| Ma | 5.8 | 4.7 | 1.1 |
| C44978 | 4.6 | 5.1 | −0.5 |
| C32352 | 4.1 | 4.1 | 0 |
| C44776 | 6.6 | 7.2 | −0.6 |
| Average | | | 0.6 |

TABLE IV

*SPI and BEI of hypothyroid patients taking thyroid*

| Subject | Thyroid | Lugol's solution | SPI | BEI | SPI−BEI |
|---|---|---|---|---|---|
| | *gm./day* | *gtts./day* | γ % | γ % | γ % |
| A79490 | 0.06 | | 3.3 | 2.4 | 0.9 |
| C9886 | 0.13 | | 6.0 | 5.4 | 0.6 |
| 48533 | 0.13 | | 4.3 | 3.9 | 0.4 |
| B7257 | 0.13 | | 4.5 | 5.0 | −0.5 |
| La | 0.13 | | 3.6 | 4.1 | −0.5 |
| B9007 | 0.13 | | 4.2 | 3.2 | 1.0 |
| 18730 | 0.13 | | 6.9 | 6.6 | 0.3 |
| C45900 | 0.40 | | 6.0 | 5.4 | 0.6 |
| | 0 | | 3.3 | 3.2 | 0.1 |
| | 0.20 | | 3.0 | 3.6 | −0.6 |
| B44180 | 0.33 | | 11.0 | 8.9 | 2.1 |
| C40160 | 0.06 | 15 | 9.9 | 6.2 | 3.7 |
| A7885 | 0.17 | 14 | 10.8 | 5.1 | 5.7 |
| | 0.17 | 14 | 13.4 | 6.6 | 6.8 |

ism. The last group of five contains, in order, two young males with obesity and a woman who were suspected of hypothyroidism, a woman five or six months after thyroidectomy, and an elderly male with cerebral arteriosclerosis. The analyses in every case were made to exclude hypothyroidism. There was not satisfactory evidence in any one of them to establish this diagnosis. With one exception, B9489, mentioned above, BEI and SPI differed by no more than 2.0 gamma per cent. Again the differences are not correlated with the concentrations of iodine. BEI is usually lower than SPI, the average value of SPI – BEI being 0.6 gamma per cent.

*BEI and SPI in hypothyroidism.* From Table IV it can be seen that BEI and SPI of patients with hypothyroidism receiving thyroid, with one exception, differed by no more than 1.0 gamma per cent; while in three patients who were also receiving inorganic iodine the differences were 3.7, 5.7 and 6.8 gamma per cent. SPI always exceeded BEI.

*BEI and SPI of patients with hyperthyroidism on medical treatment.* In contrast to the data al-

TABLE V

*SPI and BEI of patients with hyperthyroidism on medical treatment*

| Subject | Dosage for six weeks prior to study | | SPI | BEI | SPI−BEI | Pulse | Serum cholesterol | Clinical impression |
|---|---|---|---|---|---|---|---|---|
| | Lugol's solution | Propylthiouracil | | | | | | |
| | *gtts./day* | *mg./day* | γ % | γ % | γ % | | *mg.* % | |
| | | | | Hyperthyroid | | | | |
| A81700 | 14 | 200 | 15.9 | 14.1 | 1.8 | 76 | | Sweating. Skin fine. Tremor 2+ |
| B20089 | 10* | 300* | 17.1 | 12.3 | 4.8 | 98 | | Sweating. Tremor 2+ |
| Sh | 10 | 0 | 10.0 | 7.8 | 2.2 | 88 | | Loss of weight |
| B37523 | 14 | 300 | 11.9 | 8.0 | 3.9 | 98 | 223 | Sweating. Tremor 2+ |
| B86525 | 14 | 300 | 10.9 | 6.4 | 4.5 | | | Cardiac irregularity |
| C26245 | 14 | 300 | 12.5 | 8.2 | 4.3 | 96 | | Skin fine |
| Pe | 15 | 150 | 13.5 | 8.0 | 5.5 | | | |
| Na | 14 | 200 | 12.0 | 8.9 | 3.1 | | | |
| B74809 | 15 | 250 | 13.6 | 8.2 | 5.4 | | | Tense |
| 68952 | 0 | 150 | 6.5 | 7.5 | −1.0 | | | ? Hyper. |
| A83086 | 0 | 100 | 8.8 | 8.2 | 0:6 | 92 | | ? Hyper. Sl. weight loss |
| | | | | Hypothyroid | | | | |
| C36063 | 14 | 300 | 12.4 | 2.2 | 10.2 | | 584 | Puffy dry skin. Hoarse voice |
| | 14 | 0 (4 wks.) | 11.8 | 2.3 | 9.5 | | 419 | Looked less hypothyroid |
| B59207 | 14 | 200 | 7.7 | 2.1 | 5.6 | | | Puffy skin. Hoarse voice |
| | 14 | 0 (2.5 wks.) | 10.1 | 0.9 | 9.2 | | 409 | Cold dry skin |
| | | | 12.6 | 0.9 | 11.7 | | 398 | Looked less hypothyroid |
| An | 14 | 0 (2 wks.) | 5.9 | 3.1 | 2.8 | 66 | | Sleepy. Wt. gain. Sl. increase in exophthalmos |
| C38870 | 14 | 200* | 6.5 | 3.2 | 3.3 | | 217 | |
| | 14 | 0 (2 wks.) | 5.6 | 3.3 | 2.3 | | | |
| B57397 | 0 | 300 | 2.5 | 2.3 | 0.2 | 72 | 265 | |
| B92499 | 16 | 0 | 9.8 | 6.0 | 3.8 | 72 | 214 | ? Hypo. Hair normal. Skin sl. coarse. Enlarged goiter |
| De | 15 | 0 | 7.4 | 2.1 | 5.3 | | | ? Hypo. Large goiter |

TABLE V—*Continued*

| Subject | Dosage for six weeks prior to study | | SPI | BEI | SPI − BEI | Pulse | Serum cholesterol | Clinical impression |
|---|---|---|---|---|---|---|---|---|
| | Lugol's solution | Propylthiouracil | | | | | | |
| | *gtts./day* | *mg./day* | γ % | γ % | γ % | | *mg.* % | |
| Euthyroid | | | | | | | | |
| C38870 | 14 | 0 (4 wks.) | 6.6 | 3.4 | 3.2 | | 249 | |
| | | | 9.4 | 4.6 | 4.8 | | 297 | |
| 12966 | 14 | 150 | 11.4 | 4.3 | 7.1 | 80 | | Sl. weight gain |
| | 14 | 150 | 5.7 | 1.5 | 4.2 | | | |
| Bar | 15 | 100 | 9.2 | 4.5 | 4.7 | | | |
| A59918 | 10 | 400 | 12.3 | 7.1 | 5.2 | 96 | | Sweating. Tremor 2+ |
| B37523 | 15 | 300 | 7.8 | 3.1 | 4.7 | 88 | 280 | Feels better than ever before |
| 31298 | 14 | 300 | 10.0 | 6.5 | 3.5 | | | |
| | 14 | 300 | 8.5 | 4.0 | 4.5 | | | Constant weight |
| Bo | 15 | 50 | 11.5 | 4.8 | 6.7 | | | |
| B86525 | 14 | 300 | 7.2 | 5.9 | 1.3 | | | |
| | 14 | 0 (3 da.) | 7.8 | 4.5 | 3.3 | | | 3 days after thyroidectomy |
| | 0 (4 da.) | 0 (1 wk.) | 4.9 | 3.5 | 1.4 | | | 1 week after thyroidectomy |
| C20773 | 14 | 0 | 7.2 | 4.0 | 3.2 | | | |
| 51251 | 15 | 50 | 9.2 | 4.4 | 4.8 | | | Asthmatic heart disease |
| A91311 | 10 | 150 | 10.2 | 3.7 | 6.5 | | | |
| | 10 | 150 | 13.7 | 4.7 | 9.0 | 72 | | Constant weight |
| A37787 | 5 | 150 | 14.8 | 8.6 | 6.2 | 80 | | Enormous goiter |
| | 5 | 150 | 17.8 | 7.7 | 10.1 | 68 | | Sl. weight gain |
| B82863 | 14 | 100 | 7.5 | 3.7 | 3.8 | 72 | | Skin normal |
| Sc | 7 | 100 | 10.6 | 3.6 | 7.0 | 80 | | |
| | 7 | 200 | 10.6 | 6.0 | 4.6 | 78 | | Working without fatigue<br>Adenoma seems smaller |
| Sco | 8 | 150 | 6.2 | 3.2 | 3.0 | | | |
| Mi | 15 | 150 | 10.0 | 6.0 | 4.0 | | 140 | Lipiodol 2 yrs. previously euthyroid |
| C40379 | 14 | 0 | 7.2 | 4.6 | 2.6 | 72 | | |
| | 0 | 0 | 7.1 | 7.4 | −0.3 | 85 | | ? Sl. hyper. Fine tremor |
| A44295 | 0 | 200 | 10.7 | 8.0 | 2.7 | 78 | | Weight constant |
| C1807 | 0 | 100 | 7.3 | 6.6 | 0.7 | | | Hypertensive CV disease<br>? Pernicious anemia |
| C2869 | 0 | 100 | 5.8 | 4.8 | 1.0 | | | |
| Sh | 10 | 100 | 5.8 | 5.7 | 0.1 | | | |
| Pregnant patients with hyperthyroidism | | | | | | | | |
| A2544 | 15* | 300* | 15.1 | 9.5 | 5.6 | | | Vomiting. Euthyroid |
| | 15 | 300 | 11.2 | 6.7 | 4.5 | | | Euthyroid. No sweating |
| | | | 8.6 | 6.1 | 2.5 | | | |
| B94697 | 15 | 25 (3 wks.)<br>s-urea | 10.5 | 7.7 | 2.8 | | | Euthyroid |
| | 15 | 25 | 11.1 | 6.8 | 4.3 | | | Euthyroid |
| C24644 | 8 | 75 | 9.1 | 7.6 | 1.5 | 84 | | Euthyroid |
| | 8 | 100 | 8.9 | 7.2 | 1.7 | | | Euthyroid |
| 98175 | 15 | 450 | 9.4 | 6.9 | 2.5 | | | Euthyroid |
| | 15 | 450 | 14.5 | 13.7 | 0.8 | | | Actively hyperthyroid |
| | 14 | 500 (2 wks.) | 8.0 | 5.1 | 2.9 | | | Euthyroid |
| | 14 | 300 | 8.6 | 6.4 | 2.2 | | | Euthyroid |
| | 14 | 150 (2 wks.) | 14.9 | 12.9 | 2.0 | | | Hyperthyroid |

* Taken irregularly.

ready shown are wide discrepancies between 64 simultaneous measurements of SPI and BEI on 40 patients receiving antithyroid drugs for hyperthyroidism (Table V). The difference between SPI and BEI may amount to 10 gamma per cent. In only 11 of the 64 instances was the difference as little as 2.0 gamma per cent, the maximum difference in subjects not on Lugol's solution and thio drugs. The normal range of SPI is between 3.8 and 7.8 gamma per cent (6). Often SPI is well above this when BEI lies in the euthyroid range or at lower values suggestive of hypothyroidism. The euthyroid range for BEI has not really been established but has been considered to be 3.2 to 7.2 gamma per cent, 0.6 lower than the SPI range, because the average difference between the SPI and BEI of the six euthyroid subjects and of the 16 patients not on medication was 0.6 gamma per cent.

The clinical status of the patients in Table V was evaluated by a clinician when the blood was drawn. These evaluations have been used to classify the condition of these patients who were receiving medication for hyperthyroidism into the hyper-, hypo-, and euthyroid groups. In many instances only the final clinical impression has been included in the patient's history so that it is impossible to include notes on pulse, tremor, texture of skin and hair for every determination. After classification of clinical status the values for SPI and BEI were entered in the table.

All three patients who were still hyperthyroid according to pulse, tremor, etc., had SPI's and BEI's above the euthyroid range. Eight values on patients with mild hyperthyroid symptoms were above the normal range for SPI and BEI with one exception in each type of iodine determination. Eleven measurements on hypothyroid and questionable hypothyroid subjects were above or within the normal range for SPI, except for B57397, but below or at the lower limit of the range for BEI with one exception, B92499. The hypothyroid symptoms of B92499 were not classical and her serum cholesterol of 214 mg. per cent is compatible with euthyroidism. Elevation of serum cholesterol to values between 398 and 584 mg. per cent confirmed the diagnosis of the patients classified as definitely hypothyroid. Of the 30 measurements when patients appeared euthyroid, 25 BEI's were between 3.1 and 7.1 gamma per cent, but only 14 SPI's were between 3.8 and 7.8 gamma per cent. The BEI, therefore, appears to be a more accurate measure of thyroid activity than the SPI.

This conclusion is verified but is not as obvious in the values of the last group of Table V where are listed 11 values on four hyperthyroid patients who were pregnant and one value on B94697 two days postpartum. Except on two occasions of thyroid overactivity (98175) these patients appeared to be euthyroid. In euthyroid pregnancy the SPI is elevated to 6.0 to 10.0 gamma per cent (17). The SPI correlated closely with the clinical state of the patient except in the first value of 15.1 gamma per cent when A2544 seemed to be euthyroid. The BEI at the time of the 10 measurements, when patients appeared euthyroid, ranged between 5.1 and 9.5 gamma per cent. The range for BEI in euthyroid pregnancy appears reasonable because the SPI of normal pregnant women not on antithyroid medication was 6.0 to 10.0 gamma per cent and the BEI was only 0.6 gamma per cent lower than the SPI of euthyroid and untreated patients. When 98175 exhibited signs of thyroid overactivity, the BEI rose to 13.7 and 12.9 gamma per cent, concentrations indicative of hyperthyroidism even during pregnancy.

## DISCUSSION

The reasons for the false high values of SPI are still being investigated.

The amount of propylthiouracil taken does not seem to be the single factor associated with the discrepancy between the SPI and BEI. For example, four of the largest discrepancies of 9.0 to 10.1 gamma per cent were observed in two patients, C36063 and B59207, who had stopped taking propylthiouracil two to four weeks earlier, and in two patients who were taking only 100 mg. of propylthiouracil daily. In contrast to these high differences are three below 2.9 of 98175 taking 450 or 500 mg. and four differences between 0.2 and 4.5 of four patients taking 300 mg. of propylthiouracil daily (B57397, C26245, 31298, B86525).

Differences between the SPI and BEI are obvious in patients taking Lugol's solution. This is especially apparent from the results on the 12 hypothyroid patients. The SPI and BEI agreed within 1.0 gamma per cent in nine determinations on eight patients taking either 1, 2, 3 or 6 grains of desiccated thyroid daily. But two patients who were taking Lugol's as well as desiccated thyroid had SPI's 3.7, 5.7 and 6.8 gamma per cent higher than their BEI. Seven of 10 patients on Lugol's but no propyl had SPI higher than BEI in amounts varying between 2.2 and 4.8 gamma per cent (Sh, B92499, An, C38870, C20773, C40379, B86525, C36063, B59207, De).

As yet we have only six values on patients receiving propylthiouracil but no Lugol's solution. The SPI's were 0.2, 0.6, 0.7, 1.0 and 2.7 higher and 1.0 gamma per cent lower than the BEI (A44295, B57397, 68952, A83086, C2869, C1807).

The dosage of Lugol's solution does not seem to be the factor which affects the increment between the SPI and BEI. Two patients on only 5 drops of Lugol's daily had differences between the SPI and BEI of 3.8 and 6.2 gamma per cent, while two patients on the large amount of 30 drops daily had differences of 3.8 and 7.4 gamma per cent. In 40 comparisons of SPI and BEI of patients taking 14 or 16 drops of Lugol's daily, the differences ranged from 0.8 to 10.2 gamma per cent.

The difference between the values for SPI and BEI is not the result of deterioration of serum since the BEI of two hyperthyroid patients on Lugol's and propylthiouracil was the same immediately after the blood was drawn, two, and four or five days later. In one serum the SPI was 15.9, the BEI 14.1, 14.0 and 13.5 gamma per cent. In the other blood the SPI was 11.2 and the BEI 6.7, 6.2 and 6.9 gamma per cent immediately, two and five days after the blood was drawn.

The differences between the SPI and BEI of patients on Lugol's solution can not be ascribed merely to inclusion of inorganic iodine with the precipitated serum protein. SPI has been measured before and after the addition of potassium iodide to three different sera from patients not taking inorganic iodine. Two of these fresh sera were incubated at 37° for 24 hours with 200 gamma per cent of potassium iodide. In the third experiment a similar quantity of iodide was added immediately prior to precipitation with zinc hydroxide. The concentration of 200 gamma per cent of potassium iodide had been selected because this amount or less has been observed in sera of patients on 15 to 30 drops of Lugol's daily. In each instance the SPI of the serum alone was lower than that of the serum plus iodide but the differences were 0.8, 0.8, and 1.1 gamma per cent, amounts far smaller than the differences between the SPI and BEI of the patients on Lugol's solution. For example the SPI of a patient taking 30 drops of Lugol's solution (serum inorganic iodine 225 gamma per cent) was measured in two samples of serum centrifuged four times with double distilled water and on two samples centrifuged five times. The average of those washed four times was 11.8, of those washed five times 11.3 gamma per cent in spite of a BEI of 4.4 gamma per cent. No inorganic iodine could be measured in the fifth washing and only 0.3 gamma per cent in the fourth. Danowski's observations (8, 9) are in agreement with our conclusion that the presence of inorganic iodine in serum would not in itself cause the elevation of SPI above the BEI of patients taking inorganic iodine.

It seems rather that when inorganic iodine is taken, some iodine-containing compounds in addition to thyroxine are included in the SPI. This hypothesis depends on the observation that the metabolic status of patients correlated more closely with the value for butanol-extractable iodine than with that of the SPI. Our data give no evidence of the exact chemical composition of the non-calorigenic iodine compounds included with the SPI. Diiodotyrosine is a suspect since it has been demonstrated that this compound is not entirely removed from serum proteins in the procedure for the determination of SPI (16). Monoiodotyrosine and other organic compounds containing iodine may have similar affinities for the proteins (15, 18–27).

Obviously SPI may give falsely high values for circulating thyroid hormone in patients who are receiving inorganic iodine. The conclusion has been drawn that a SPI as high as 8.0 gamma per cent is compatible with euthyroidism, although the average value for normal subjects is 5.4 gamma per cent. Engstrom and coworkers (7), in a critical analysis of the SPI of 139 hyperthyroid patients under medical treatment, found that SPI agreed with clinical criteria in 119, or 86 per cent. Discrepancies were encountered most frequently when patients became slightly or frankly hypothyroid (5). In these cases SPI did not fall below 4 gamma per cent.

Investigations are continuing to ascertain whether, as seems probable, BEI is a more accurate measure than SPI of circulating thyroid hormone.

The artifactual high values of SPI produced by priodax are also found in BEI.

## CONCLUSIONS

SPI and butanol-extractable iodine (BEI) differ by no more than 2.0 gamma per cent in normal persons, pregnant women, and persons with thyroid disorders who are untreated or receiving thyroid. SPI usually exceeds BEI by an average of 0.6 gamma per cent.

In patients who have received inorganic iodine, SPI frequently exceeds BEI. The BEI seems to be more consistent than SPI with other criteria of the clinical status of such patients.

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# PERIPHERAL VASCULAR HEMODYNAMICS IN THE BULBAR CONJUNCTIVA OF SUBJECTS WITH HYPERTENSIVE VASCULAR DISEASE [1, 2, 3]

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(Submitted for publication October 11, 1950; accepted, March 19, 1951)

Within recent years, methods have been developed for direct observational studies concerned with functional aspects of the muscular vessels in the capillary bed of living small mammals of several species (1–3). With these technics, it has been found that certain objective criteria of peripheral vascular reactivity can be selected and followed during repeated examinations of the terminal vessels with the microscope. Comparisons of the several different overall hemodynamic disturbances in the experimental animal (*i.e.*, hypertension, acute hemorrhage, prolonged hypotension) with their co-existent phenomena in the peripheral vascular system have shown that each separate condition thus far investigated has had a distinctive status reflected in the capillary bed of the mesentery. For example, acute hemorrhage was accompanied by widespread arteriolar and metarteriolar constriction, capillary ischemia, and poor venular return. The reactivity of metarterioles and precapillary sphincters to topically applied epinephrine was augmented. Terminal prolonged hemorrhagic or traumatic hypotension, on the other hand, had a dilated capillary bed, with sluggish arteriolar and venular blood flow, plethoric capillaries and diminished epinephrine responses (2, 4, 5). The mesenteric vascular bed in experimental renal hypertension was predominantly constricted, with ischemic arterioles and capillaries and a similar elevation of epinephrine response. In addition, it had a marked "hyperplasia" of vascular elements (6).

It should be emphasized that no *single* feature of those which were selected to be followed in animals could be described as "characteristic" of any of these experimental syndromes. Vasoconstriction, or elevated epinephrine responses, or sluggishness of blood flow, etc., were often found in various and differing situations. The specificity of the hemodynamic state became apparent only when all of the functional criteria used were considered together.

The observational methods and technics of eliciting peripheral vascular responses to stimuli during examinations of the vessels with the microscope have been adapted, with modifications, to the study of metarterioles, capillaries and collecting venules in the conjunctiva of man. The description of the microscope and apparatus, which are comparable to those used by others (7), together with the procedures and observations on young adults without known disease, have been previously published (8). Since then the survey has been extended to include patients with hypertensive vascular disease. It is the purpose of this report to describe certain peripheral vascular hemodynamic responses in these patients and to compare them with findings in normotensive subjects.

## METHODS

The technics employed in this study were those used and described in the previous report (8). In brief, the bulbar conjunctiva in each eye was examined with a slit-lamp microscope in a manner similar to that used in routine ophthalmological practice. After establishing that conjunctival disease *per se* was absent, the general state of the capillary bed was noted (*i.e.*, ischemic, plethoric, euvascular, etc.). In certain cases, the velocity of blood flow was measured in the metarterioles, capillaries and collecting venules with the method previously described (8). This procedure consisted of repeated determinations with an ocular micrometer in the microscope, and a fast stopwatch, of the durations of the passage of red

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[1] This work was supported in part by the Nutrition Foundation, New York, N. Y.

[2] Apparatus used for the study of the conjunctiva was obtained through a grant in aid from the American Philosophical Society, Philadelphia, Pa.

[3] The authors gratefully acknowledge the stimulating interest and encouragement of Dr. Ephraim Shorr and Dr. Benjamin William Zweifach.

*blood cells over a measured length of vessel. At the magnifications used (47 ×), single red blood cells, and cell aggregations* can readily be seen and followed. In measurement of flow speed, vessels were selected (arterioles, capillaries and venules) that were as straight as could be found, over a distance of at least 75–100 microns. After a minimum of practice, the method has been found to furnish comparable data with different observers.

In a total of 30 subjects, the incidence of spontaneous vasomotor activity [1] in the precapillary sphincters was estimated. Metarteriolar and precapillary sensitivity to directly applied epinephrine in known concentration was then measured. It proved important to begin with the most diluted solution (1 : 800,00–1 : 400,000 epinephrine) and use progressively stronger concentrations (at intervals of three minutes) until the threshold precapillary constrictions were obtained. This avoided possible sensitization of the vascular smooth muscle cells by repeated exposure to epinephrine solutions of greater than threshold strength. A vasoconstrictor response to cold can be measured by applying an ice pack to the closed lid for progressively greater intervals until precapillary constriction is seen. This type of response may also be tested by routine use of a short period of exposure to ice (10 seconds) which is without subsequent vascular change in a large series of controls, yet which is followed by vasoconstriction in certain disease states (cirrhosis, multiple myeloma). The latter method was employed in this study.

To date, examinations of the bulbar conjunctiva in hypertensive individuals have been made on 14 men and 31 women. The mean age was 31 years, with age distribution from three to 78 years.[5] During repeated studies over periods of from two to three months, the blood pressures were consistenly greater than 140/100 in all subjects. Only those cases were selected who had no history of renal or hepatic disease or cardiac incompetency. P.S.P. tests, urine concentrating and diluting capacities and blood urea nitrogen concentrations in all cases demonstrated no renal insufficiency. Intravenous pyelograms were carried out on 20 subjects and retrograde pyelograms were made on three others. These X-rays revealed no evidence of renal abnormality.

In addition, similar conjunctival observations were made on 33 patients with various normotensive systemic diseases, such as multiple myeloma, cirrhosis, peptic ulcer, polycythemia; and with psychosomatic disorders. None of them had a history of hypertension. These cases had received a wide variety of clinic and laboratory studies. Those of each subject were appropriate only to his specific illness. The vascular findings in this group are therefore representative of a random sampling in a diseased population and are included only for the purpose of comparing their variability with the relatively consistent phenomena displayed by the blood vessels of the patients with "essential hypertension." The results of the survey on 72 normotensive subjects without evidence of disease are also included in the data.

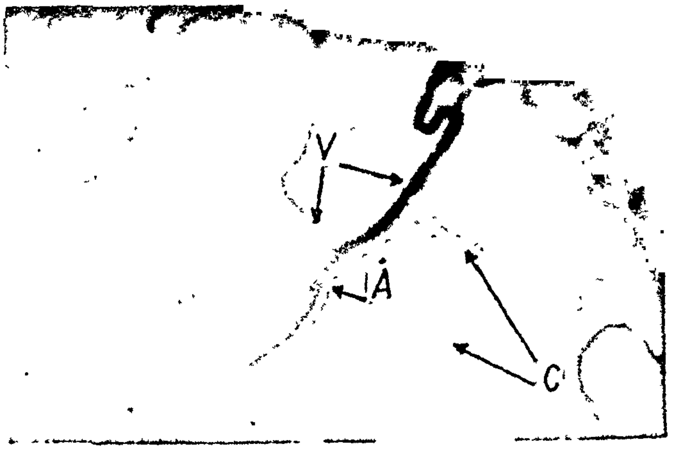

FIG. 1. THE CONJUNCTIVAL CAPILLARY BED OF A NORMOTENSIVE SUBJECT WITHOUT DISEASE

A = terminal arteriole, from which the true capillaries (C) arise. These latter vessels are of uniform caliber and in general follow a smooth, sinuous pathway over the conjunctiva. Two capillaries in the right half of the picture, however, are slightly tortuous or coiled. The collecting venules (V) drain the capillaries, then form larger venules which are frequently situated approximate to the arteriole, as shown. The small venules in the left portion of the picture display the venular loopings which are not uncommon in normotensive individuals (120 ×). (This is another photograph of the same field shown in Figure 1 of the previous publication.)

## RESULTS

The capillary bed and its reactions in the conjunctiva of normotensive subjects has been previously described (8). Its topographical arrangement in shown in Figures 1 and 2. The terminal arterioles, usually accompanying larger collecting venules, frequently extend radially from the orbital margins. Capillaries arise from these vessels to form the blood capillary network of the conjunctiva. These vessels become confluent to form the most peripheral system of collecting venules, which in turn empty into the larger venules accompanying the nutrient arterioles (Figure 1).

The general nature of the circulatory state in the terminal vascular bed in hypertension was that of overall vasoconstriction. Metarterioles were notably more narrowed than those of controls; and occasionally they were actually smaller in diameter than were the true capillaries. This latter phenomenon is well known in lower animals and is believed to be the basis for earlier descriptions of "capillaries" (*i.e.*, the smallest terminal vessels) as elements with contractile (muscle) cells along the endothelial wall. The increased narrowing was variable in different subjects, ranging from a difference noted only in two to three arterioles of each conjunctiva to a widespread vasoconstriction with pallor and closure of most of the precapillary sphincters. In general, the degree of vascular narrowing was correlated with the level of the diastolic pressure, *for it was more pronounced in subjects* with the greater diastolic elevations. It was noted also to be more severe, however, in patients with a hyperkinetic reaction pattern during interview

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[1] Vasomotion is an apparently spontaneous variation in caliber resulting from contraction and relaxation of the vascular smooth muscle. It is usually irregular in rate, in degree and in duration of dilated and constricted phases.

[5] The Department of Pediatrics at Cornell University Medical College and The New York Hospital has been very interested and cooperative in this study. The authors are very grateful to the department, and to Dr. Sarah Matteson, for alerting them to the occasional occurrence of "essential hypertension" in early childhood, and for their cooperation in the project.

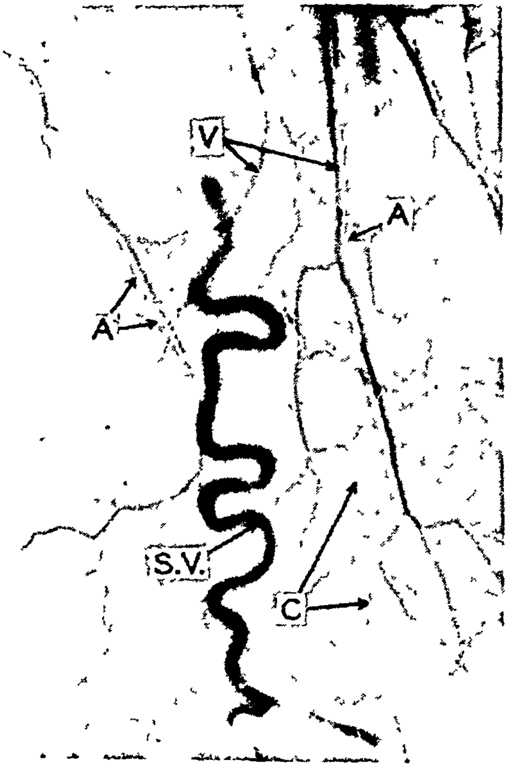

FIG 2 LEFT TEMPORAL CONJUNCTIVA IN NORMOTENSIVE SUBJECT WITHOUT OTHER DISEASE, R L, AGED 35

The metarterioles (A) are usually straight and are approximately one-half the diameter of the accompanying venules (V) The true capillaries are seen here as fine slightly curved lines forming a regular pattern between the larger vessels (80 ×) (S V = scleral vein, commonly of this tortuosity)

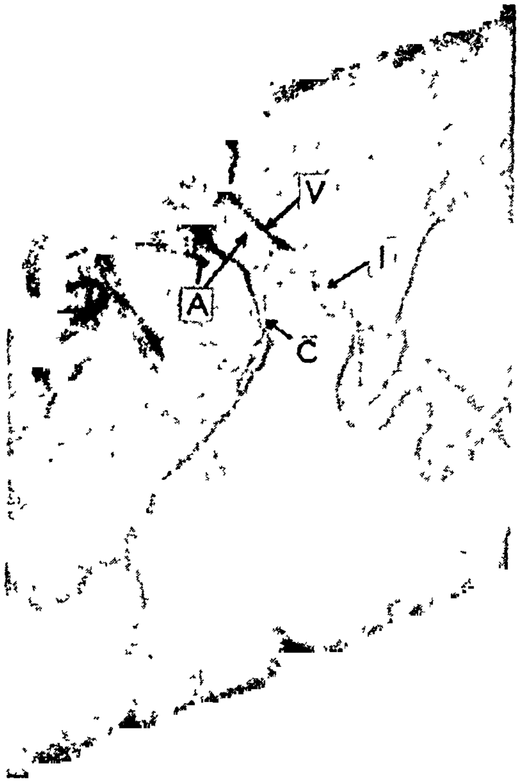

FIG 3 LEFT TEMPORAL CONJUNCTIVA IN HYPERTENSIVE SUBJECT WITHOUT OTHER DISEASE, G H, AGED 3

The metarteriole (A) and the collecting venule (V) are moderately sinuous The metarteriole (A) is completely looped (1), in contrast to the straight arteriolar pathway shown in Figure 2, and one capillary shows an irregularly serrate course (C) (80 ×)

The vasoconstricted state was associated with a reduced velocity of blood flow in all observed arterioles, capillaries and venules This was most readily seen in the true capillaries and collecting venules

In 15 hypertensive patients, the occurrence of spontaneous vasomotor activity at precapillary sphincters was compared to that found in 12 control individuals and 12 patients with normotensive disease In both normotensive and hypertensive *subjects vasomotion varied in rate and in duration* of the dilated and the constricted phases. In the hypertensives, in contrast to findings in all other subjects, it was more rapid with relatively long periods of precapillary narrowing. This resulted in intermittent, or interrupted, capillary blood flow. A sudden jet-like column of red cells would enter the capillary and flow would be uniform for several seconds. The stream was then gradually reduced as the precapillary narrowed, with subsequent longer periods during which only scattered red cells were admitted into the capillary from the parent arteriole.

The minimum concentration of epinephrine which produced threshold constrictions of the precapillaries and metarterioles in hypertensive patients was approximately 1/10 the threshold strength necessary to produce constriction in similar vessels of normotensive individuals without other disease. A comparable elevation of the vascular reactivity to epinephrine has been observed to date only in cases of Laennec's cirrhosis and in patients with hyperglobulinemia. Subjects with these abnormalities constituted 45% of our control

TABLE I

*Peripheral vascular findings in normotensive and in hypertensive subjects*

| | *Group I*<br>Normotensive subjects without known disease<br>*(72)* | | *Group II*<br>Hypertensive vascular disease<br>*(45)* | | *Group III*<br>Normotensive subjects with various and differing diseases<br>*(33)* | |
|---|---|---|---|---|---|---|
| General nature of the vascular system | Vessels tonic; flow smooth and rapid; no stagnation | | Vessels narrowed; tissue ischemic; flow slowed | | Variable, from wide dilation and sluggish flow, to euvascular | |
| Spontaneous vasomotor activity | *(12)*<br>Present up to grade 1 in 3 of 12 cases; variable in duration and frequency | | *(15)*<br>To grade 3–4 in 11 of 15 cases; rapid; "closed" phase predominated | | *(12)*<br>Usually not seen; highly variable | |
| Velocity of peripheral blood flow (mm./sec.) | *(10)* | | *(10)* | | *(10)* | |
| | *Mean* | *S.E.* | *Mean* | *S.E.* | *Mean* | *S.E.* |
| End arterioles | 0.11 | 0.03 | 0.023 | 0.004 | 0.034 | 0.010 |
| Capillaries | 0.026 | 0.002 | 0.010 | 0.001 | 0.008 | 0.004 |
| Venules | 0.056 | 0.005 | 0.018 | 0.001 | 0.016 | 0.006 |
| | | | | | Greatly reduced in all vessels in cases of hyperglobulinemia and some cases of cirrhosis | |
| Coiling, tortuosity and looping of vessels | Up to grade 1 in 8% of subjects | | Grade 3–4 in 62% of the cases<br>Grade 1–2 in 12% of cases | | Up to grade 1 in 18% of subjects | |
| Epinephrine concentration producing threshold pre-cap. and metarteriolar narrowing | *Mean*—1:37,000<br>*S.E.*—1:03,670<br>*Range*—1:20,000–1:50,000 | | *Mean*—1:298,000<br>*S.E.*—1:24,700<br>*Range*—1:100,000–1:800,000 | | *Mean*—1:314,000<br>*S.E.*—1:32,000<br>*Range*—1:10,000–1:800,000<br>Elevated above group 1 mean only in Laennec's cirrhosis and hyperglobulinemia to date | |
| Reaction to cold ice pack application (15 sec.) | none | | Grade 1 in 7<br>Grade 2 in 1 | | Variable, from no response to a grade 4 vasoconstriction, with stagnant flow and red cell aggregates, in patients with hyperglobulinemia | |
| Effect of prior cold exposure on epinephrine sensitivity | none | | none | | Increased in 5 cases of hyperglobulinemia | |

The figures in parentheses indicate the number of patients studied. Unless otherwise indicated, determinations were carried out on all subjects. Spontaneous vasoconstriction, the reaction to cold, and the degree of abnormal capillary topography, are graded in ascending severity from 1 to 4.

From the Table of x, the differences in mean values for velocity of flow and epinephrine reactivity between the normal subjects of Group I and the disease groups are highly significant. A comparison of these two features is difficult, however, and not quite significant, between Groups II and III, because of the great reduction in flow speed and augmentation of epinephrine reactivity that was uniformly observed only in patients with hyperglobulinemia and certain cases of Laennec's cirrhosis, in the latter group. These wide specific variations, with the extremely heterogeneous nature of Group III, encumber statistical analysis of its flow speeds and vascular reactions.

disease group. For this reason, the average vascular epinephrine reactivity in these non-hypertensive illnesses was also greatly elevated. This is in accord with the situation which obtains in the experimental animal, in that precapillary reactivity to epinephrine may be augmented in certain other states as well as in experimental hypertension.

A minimal vasoconstrictor response to topical cold (grade 1 or below) occurred in seven of 27 of the hypertensive patients. An eighth subject, a 10 year old boy, had a grade 2–3 response to this test. Twelve of 21 patients with diseases other than hypertension had elevated vascular responses to cold (to grade 4) (Table I). Vasoconstriction in response to the cold stimulus used in this series was not observed in any of the normotensive non-diseased persons. Exposure to cold before the application of epinephrine to the conjunctival vessels had no detectable effect on the epinephrine response in any of the subjects excepting a small group of five normotensive patients with multiple myeloma.

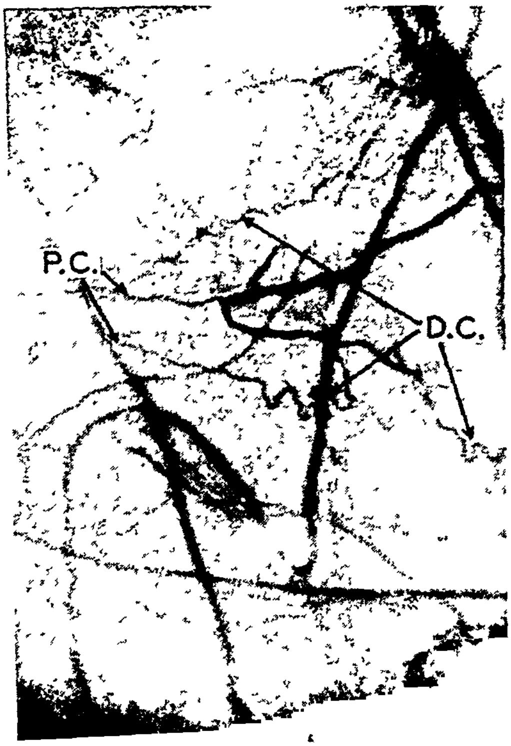

Fig. 4. Subject G H. (as Figure 3)

The capillaries are shown at higher magnification to demonstrate the uniform nature of their proximal (arteriolar) portions (PC). The distal segments of the capillaries (DC) are tortuous and slightly coiled (120 ×) These changes shown in Figures 3 and 4 not only indicate the morphological abnormalities, but also demonstrate that they may exist in very young subjects with hypertension.

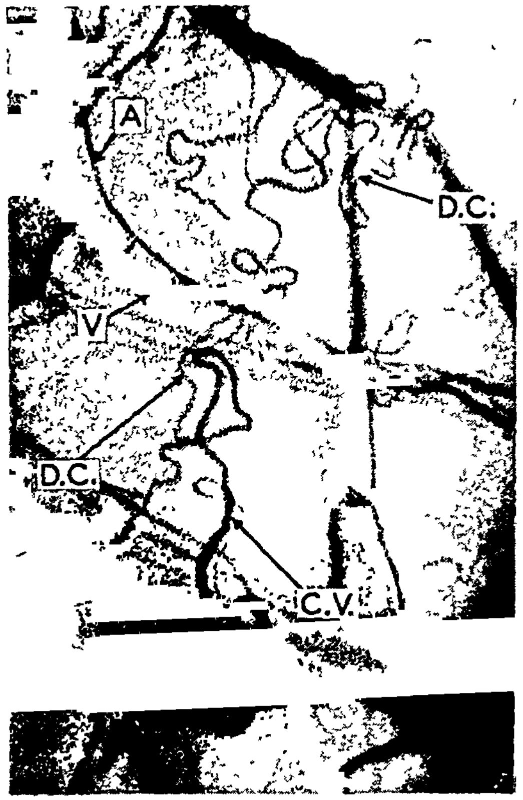

Fig 5. Left Temporal Conjunctiva in Hypertensive Subject without Other Disease, M. C, Aged 37

The larger metarteriole (A) and venule (V) are relatively uniform. The distal capillary segments (DC) and the collecting venules (CV) are excessively coiled, looped and elongated in comparison with those of normotensive subjects shown in Figures 1 and 2. (120 ×)

In 62% of the hypertensive subjects, abnormal patterns of vascular topography, of grade 3 severity or greater, were observed. These consisted of tortuosity of arterioles and accompanying venules, with occasional looping and coiling of vessels in a striking manner (Figures 3–5). The capillaries in these cases were angular, looped and often tremendously elongated; and frequently followed a tortuous pathway over the conjunctiva (Figure 5). A somewhat comparable appearance of the vessels was noted in 18% of cases with normotensive disease, with slight coiling and tortuosity in several capillary vessels. In these latter subjects, however, it was of minimal degree (grade 1 or below). This unusual vascular pattern in hypertension frequently was of considerable magnitude in the conjunctiva of one eye and much less severe in the other. Tortuosity of the retinal arterioles and venules was also noted in many of the these hypertensive cases. This abnormality was found in three of these subjects to be largely unilateral in the retina as well.

Four individuals were examined who previously had been hypertensive. They were normotensive and asymptomatic at the time of study and had been so for periods of from four months to three years. The remission of hypertension was spontaneous in each instance. Three of the subjects had had "toxic" pregnancies, while the fourth was a 35 year old male who formerly had experienced only minor frontal headaches. None had any evidence of disease. The conjunctival metarterioles, however, were moderately constricted in all four cases, and peripheral vascular epinephrine sensitivities were consistently within the "hypertensive range." In addition, the conjunctival capillaries of two of these cases were tortuous and coiled (grade 3).

## DISCUSSION

Studies of the mesenteric capillary bed in the experimental renal hypertension of rats and dogs in this laboratory have revealed certain consistent changes in the peripheral vessels. Following the proper application of Goldblatt clamps to the renal arteries or the use of perinephric silk packs, the arterial mean pressure rose gradually. Coincidentally, vasoconstriction developed in the terminal arterioles and spontaneous vasomotion became augmented (6, 9). The response of the metarterioles to topically applied epinephrine was elevated. A striking topographic feature of the peripheral vascular beds observed in this condition was the relatively greater number of vessels found per unit area of the tissues. Metarterioles, capillaries and collecting venules were all three to four times as numerous as those in the normotensive animals (6).

The changes directly observed with the microscope in the mesenteric capillary vessels of animals with renal hypertension are generally in accord with the phenomena present in the conjunctival vascular bed of hypertensive patients. The "hyperplasia" of elements in the capillary bed of the mesentery as found in the renal hypertensive animal was not noted in conjunctival vessels of hypertensive patients, as selected by the methods described above. However, the increased vascular tortuosity, vessel loops and angular capillary topography that was found in 62% of the patients may represent an analogous phenomenon. Each might conceivably be a morphological pattern of response elicited either by prolonged vasoconstriction or by an increased pressure load on the terminal arteriolar tree. The abnormal vessel patterns in hypertension have been described previously, with other phenomena and "blood sludging," by Lack and co-workers (10). We found tortuosity and looping of vessels less frequently, however, in our cases than reported by these workers and noted a somewhat similar topography of minimal degree in occasional patients without hypertension.

The presence of vasoconstriction with a reduced peripheral flow velocity in the conjunctiva of hypertensive subjects seems at variance with the concept that, in this condition, overall peripheral blood flow is not changed. Assuming that this belief is correct immediately implies that a reduction in flow speed in one region (*i.e.,* the conjunctiva or the mesentery) is compensated by a relative hyperemia with augmented peripheral flow elsewhere. However, it is conceivable that diminished velocity of *capillary* blood flow *per se* can exist, and be obscured by the shunting action of arterial and arteriolar-venular arcades that may be located just proximally to the capillary bed. Such vessels are known to exist in the splanchnic area of animals (1). Increased gaseous exchange in the slowed capillary blood may partly mask the relatively unchanged gas content in the shunted blood bypassing the capillaries. Indirect methods of estimating the volume and velocity of actual capillary blood flow might not indicate such a terminal diversion of blood from areas of end-arteriolar vasoconstriction and reduced flow velocity. These latter phenomena, therefore, may possibly be more widespread in the most peripheral vessels in hypertension than is generally believed.

In the present survey we found no absolute correlation between any *single* feature of the conjunctival vascular system and either the diastolic level of pressure, the severity of symptoms, or the duration of the hypertension. Heightened sensitivity to epinephrine occasionally was found in normotensive disease. Abnormal vessel arrangements of minor degree occurred in several diseased controls and were well advanced in a hypertensive child of three years of age who was symptom free (Figures 3 and 4). Thus these anatomical changes are not necessarily correlated in degree either with the age of the patient or the presence of symptoms. However, narrowed metarterioles, and prominent spontaneous vasomotion of a predominantly "closed" phase and tissue ischemia were the most frequent findings. They were observed in 90% of the hypertensive subjects and in none of the controls; and were frequently, but not consistently, of greatest magnitude in subjects with a diastolic pressure of 140 mm. Hg or greater. The tortuosity and coiling of vessels with angular capillary patterns was the second most usual feature.

When all of the criteria used for evaluating the conjunctival capillary vessels were considered together, a composite state of the peripheral vascular bed in hypertension evolved that was not observed in patients with other disease, or in normal subjects. This state was featured by narrowing of the most terminal arterioles, augmented spontaneous vasomotor activity, and frequent elongation, coiling, and tortuosity of the capillaries. Accompanying these phenomena was a heightened metarteriolar and precapillary reactivity to topically applied epinephrine, and a significant reduction in the velocity of peripheral blood flow. This latter was most noticeable in the true capillaries and the collecting venules. The finding that several criteria may permit establishment of an overall peripheral vascular hemodynamic pattern that is inadequately described by any one feature is in accord with the observations on the vascular bed of animals, mentioned previously.

The conjunctival vascular state during hypertension, however, apparently is related to circumstances other than the blood pressure elevation *per se,* for it was also noted in the four subjects whose hypertension has spontaneously subsided at a period considerably before the examinations were carried out. Clinically, all studies failed to distinguish these individuals from other normotensive subjects without disease, yet on a basis of the findings in the conjunctival capillary bed they could not be separated from patients with a sustained hypertension. It is of interest to speculate whether such functional peripheral vascular changes not only persist following the cessation of prolonged hypertension, but may also occur prior to its onset.

## SUMMARY

1. With suitable apparatus and techniques for measuring vessel function and reaction, studies were made of the conjunctival capillary vessels of subjects with hypertensive vascular disease.

2. When all criteria selected for evaluation were considered together, an overall pattern emerged of certain peripheral vascular abnormalities in hypertension. The total pattern was not found in the normotensive controls. No one feature selected for study could be correlated absolutely either with the age of the patient, the degree of blood pressure elevation, symptomatology or the duration of the illness. This need for a composite of several criteria to establish a peripheral vascular dysfunctional state is in agreement with findings in experimental animals.

3. The abnormal hemodynamics observed in the conjunctival peripheral circulation in essential hypertension consisted of: *1*) metarteriolar narrowing; *2*) augmented spontaneous vasomotor activity in precapillary sphincters; and *3*) coiling, looping, and tortuosity of many vessels. There was also present a heightened sensitivity of metarterioles and precapillaries to topically applied epinephrine, and a reduced velocity of peripheral blood flow. These features are generally in accord with those which have been described for similar vessels in the splanchnic area of animals with experimental renal hypertension.

4. The conjunctival peripheral vessels of four normotensive subjects who had had an apparently spontaneous remission of previous hypertension displayed the same phenomena found in the conjunctival vascular bed of patients with a sustained hypertension.

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# SENSITIVITY OF SERUM ENZYME INHIBITORS TO A VARIETY OF CANCER CHEMOTHERAPEUTIC AGENTS [1, 2]

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(Submitted for publication October 31, 1950; accepted, March 19, 1951)

In the palliative treatment of malignant disease, an objective method of determining the rapidity, degree, and duration of therapeutic response is a constant requirement. In a recent publication, Rhoads (1) has re-emphasized the importance of detecting delicate therapeutic effects in clinical cancer research. Standard methods such as serial x-rays, blood counts, blood chemistry, observations of temperature, tumor size, changes in body weight, etc., are frequently of only limited usefulness for this purpose. Without a simple and objective measurement of tumor activity, valuable days or weeks may be needlessly spent on ineffective treatment. Similarly, reactivation of the disease may be difficult to detect by conventional methods, until it has produced irreversible damage and the patient can no longer tolerate intensive therapy.

Up to the present, there have been a few specific instances in which clinical laboratory tests have been helpful in following the effects of cancer treatment, *e.g.*, serum acid phosphatase in carcinoma of the prostate, alkaline phosphatase in osteogenic sarcoma, and serum globulin levels in multiple myeloma, but these are obviously of limited application. Recently, however, it has been shown that the serum concentration of proteolytic enzyme inhibitors is influenced by various pathologic states, including malignant disease (2, 3). Although they possess no diagnostic value, two of these substances, chymotrypsin inhibitor and rennin inhibitor are nevertheless of remarkable clinical usefulness. In a study of cancer patients receiving nitrogen mustard therapy, West and associates (4) were able to relate the ratio of serum concentrations of these two factors to clinical signs of tumor activity. The present paper illustrates the sensitivity of the enzyme inhibitor response to a number of additional chemotherapeutic agents in several typical cases of neoplastic disease.

## METHODS

Patients selected for case reports are from the Neoplastic Disease Service of the Veterans Administration Hospital, Long Beach, California. Although most have been subject to prolonged hospitalization and complete clinical and laboratory studies, only condensed case summaries dealing with pertinent factors are presented.

Quantitative determinations of the two inhibitors in the serum were made by methods previously described by West and Hilliard (2). Results were correlated with clinical and other laboratory evidence of changes in neoplastic activity. In all cases the existence of cancer had been proven by biopsy, and the patients were free of complications which have been shown to influence the enzyme inhibitor concentrations. Under these circumstances, as will be demonstrated, high rennin inhibitor and low chymotrypsin inhibitor levels are found to be associated with arrested growth or tumor regression while the reverse is correlated with actively progressing disease.

## EFFECTIVE CHEMOTHERAPY

### *Urethane in myelogenous leukemia*

Since the introduction of urethane for the treatment of myelogenous leukemia by Haddow and Sexton in 1946 (5), this drug has proved to be a valuable additional agent for palliative treatment of this disease. The case presented indicates the sensitivity of serum enzyme inhibitor concentrations to effective urethane therapy.

### *Case No. 1 (Figure 1)*

A 28-year-old Mexican male was admitted to the hospital with complaints of fullness in the left upper quadrant for eight months, anorexia, weakness, fatigue, and 20 pounds weight loss of two months duration.

Abnormal physical findings included pallor, enlarged spleen extending beyond the mid-line and into the pelvis,

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[1] This work was supported in part by grants from the California Institute for Cancer Research through the Damon Runyon Cancer Fund.

[2] Reviewed in the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions published by the authors are the result of their own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

liver enlargement three fingers' breadth below the costal margin, and a few small submaxillary, axillary, and inguinal lymph nodes.

Laboratory findings included entrance white blood cell count of 420,000 with 94% neutrophiles, (two myeloblasts, nine promyelocytes, 14 myelocytes, 20 stabs, 30 juveniles), 4% eosinophiles, 2% basophiles. The red blood cell count was 2,870,000 and hemoglobin 8.4 gms. Platelets 280,000. Sternal marrow aspiration showed a typical picture of active chronic myelogenous leukemia with depression of erythroid activity.

The patient was started on oral urethane 6.0 gms. daily, which was subsequently gradually reduced to 2.0 gms. as shown in Figure 1. He improved steadily, the spleen reduced in size, hepatomegaly and lymphadenopathy disappeared, and he regained his feeling of well-being. He was discharged on maintenance doses of urethane.

Figure 1 illustrates a decrease both in white count and in chymotrypsin inhibitor activity coincident with the patient's improvement. During the charted period the hemoglobin increased from 8.4 to 13.2 gms., and the red blood cell count from 2,870,000 to 4,510,000 without transfusion of blood. Although urethane appears to have delayed action in chronic leukemia, it is of interest to note that its enzymatic effects are almost immediate and detectable many days ahead of the hematologic response. Assuming that urethane acts principally upon the immature marrow, delayed depression of the peripheral white count is not unexpected. When the white blood count had decreased from over 400,000 to 30,000, the drug was stopped, in spite of which the count continued to fall to a minimum of 10,250 11 days later. However, upon discontinuing urethane, the enzyme inhibitors returned rapidly to pre-treatment levels. This occurred 10 days in advance of the corresponding rise in white blood cell count. Because the anti-leukemic action of urethane is reflected promptly in the enzyme inhibitor pattern, it has been found that maintenance doses of the drug may be selected more quickly on this basis.

Two additional cases of chronic myelogenous leukemia, and two of multiple myeloma have been treated with urethane with essentially similar clinical and enzymatic responses. In contrast to these, other types of leukemia, and several cases of carcinoma of the colon and prostate that received urethane without clinical improvement, failed also to show any change in the concentration of serum enzyme inhibitors.

*Cortisone in lymphosarcoma*

Rapid regression of lymphosarcoma following administration of cortisone was first observed by Heilman and Kendall (6) in tumor bearing mice.

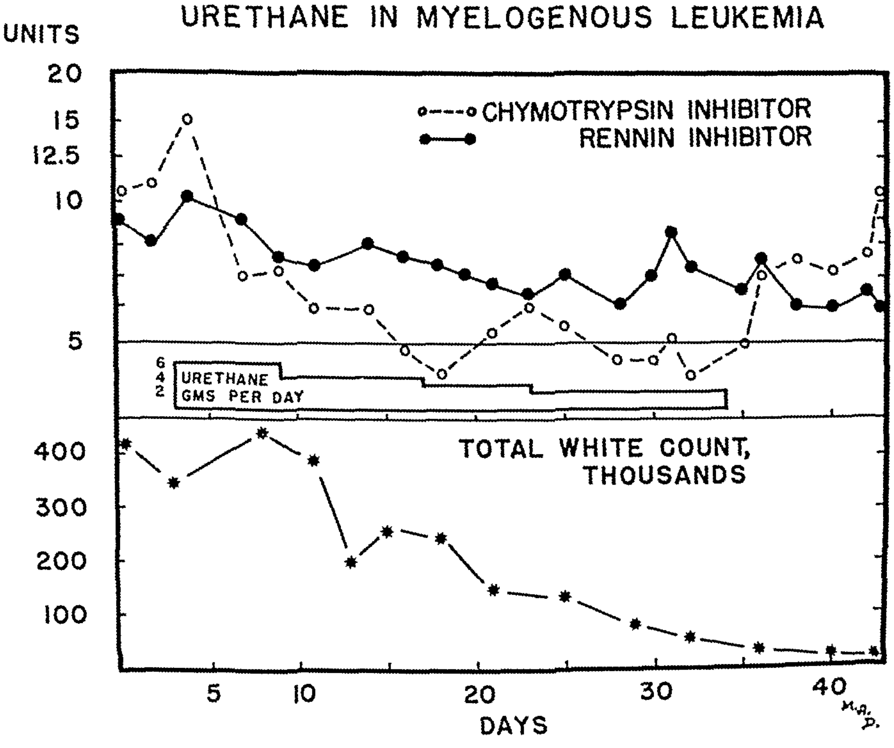

FIG. 1. ILLUSTRATING THE SENSITIVITY OF CHYMOTRYPSIN AND RENNIN INHIBITORS TO THE ANTI-LEUKEMIC ACTION OF URETHANE

More recently, Pearson and associates (7) have demonstrated that chronic lymphomas in man may respond similarly both to cortisone and ACTH. Although results are admittedly of a temporary nature, nevertheless cortisone-induced regression of lymphosarcoma and the subsequent regrowth of the tumor provide an interesting test of the sensitivity of the two serum inhibitor substances to neoplastic activity.

*Case No. 2 (Figure 2)*

This 42-year-old white male had been followed by the tumor service since February 1949 when he was first hospitalized for bilateral groin swellings. Initial examination showed multiple inguinal, axillary, cervical, and occipital nodes. Biopsies of both inguinal area, and later of axillary nodes showed giant follicular lymphoblastoma, lymphosarcomatous type. Patient received x-ray therapy in March–April 1949; nitrogen mustard in October 1949; second course of x-ray in December–January 1950; third x-ray course March 1950. He always responded well, maintained excellent physical condition, and was followed enzymatically at twice-monthly intervals, his satisfactory clinical course being mirrored in his favorable enzyme pattern.

When seen on May 22, 1950 he complained of anorexia, seven pound weight loss, low-grade fever, and enlargement of inguinal nodes. Chymotrypsin and rennin inhibitor levels at this time also suggested renewed activity and the patient was advised of the need for re-treatment.

On admission he complained of rather severe left flank and lumbar pain and showed multiple small cervical and occipital nodes, and discrete right inguinal nodes measuring 2 to 3 cm. Laboratory work was within normal limits, as on multiple previous determinations: red blood cell count 4,510,000, hemoglobin 13.2 gms., white blood cell count 5,650 with 62% neutrophiles, 26% lymphocytes, 8% monocytes, 5% eosinophiles. No abnormal white blood cells were seen. X-rays of the lumbar spine and abdomen showed no abnormalities.

The patient received cortisone, total of 1.0 gm. in seven days. Within 24 hours the back and flank pain had entirely disappeared, and the nodes were smaller. Within three days the cervical and occipital nodes had vanished, and the inguinal nodes were much reduced. He gained weight, felt entirely well, and returned to his job as truck driver.

Figure 2 illustrates a rapid decrease in serum chymotrypsin inhibitor immediately after cortisone[3] was started. This change was coincident with tumor regression and general clinical improvement. Also demonstrated is the transient effect of cortisone in this case, with relapse within three weeks. The patient was later treated with ACTH for a similar period with excellent but equally temporary improvement both clinically and enzymatically. Because cortisone therapy did not result in an increased rennin inhibitor titer, it was predicted that the remission would be short, since in the course of this work it has been observed that prolonged remissions are usually associated with combined high rennin and low chymotrypsin inhibitor levels. For example, when nitrogen mustard was given to this same patient later, there was an increase in rennin inhibitor as well as a fall in chymotrypsin inhibitor, and more lasting clinical results were obtained.

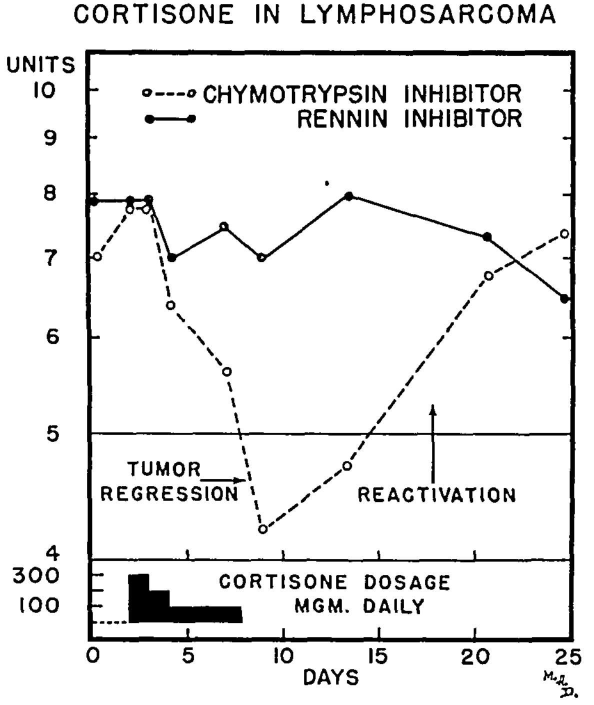

FIG. 2. REDUCTION OF SERUM CHYMOTRYPSIN INHIBITOR COINCIDENT WITH CORTISONE-INDUCED REGRESSION OF LYMPHOSARCOMA

*ACTH in acute leukemia*

The clinical investigations of Farber and colleagues (8) and Pearson and coworkers (9) indicate that incomplete and temporary remissions in acute leukemias of both children and adults can be induced with considerable regularity by the use of ACTH. With the dosage schedules that have been employed, however, the majority of adults have been refractory to subsequent therapy. In the case presented below, the parallelism between hematologic improvement and changes in the enzyme inhibitors was studied by giving the drug intermittently in small amounts. Under these conditions repeated responses to ACTH[4] were observed and the enzyme inhibitor pattern proved to be the most sensitive available index of disease activity.

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[3] Cortone acetate, Lot No. 415, Merck and Co., Inc.

*Case No. 3 (Figure 3)*

A 38-year-old white male was admitted to the hospital with complaints of pain in the left upper abdomen, profuse night sweats, weight loss, anorexia, all of one month's duration, and tarry stools and ankle edema, for two weeks.

Abnormal physical findings on entry included severe pallor, scattered petechiae on buccal mucosae, spleen enlarged to the level of the umbilicus, hepatomegaly of two fingers' breadth, and numerous bean-sized firm inguinal nodes.

Laboratory findings included white blood cell count of 18,900 with differential of 65% blast cells, unclassified, 12% neutrophiles, 25% lymphocytes. Red blood cell count 2,810,000, hemoglobin 8.1 gms. Platelets 210,000. Sternal marrow aspiration showed 95% blast cells, confirming diagnosis of acute blastic leukemia. Subsequently cells of the myeloid series became prominent, particularly high percentages of myeloblasts and myelocytes. Total white blood cell count varied from 6,000 to 94,000 with percentage of blasts from 17% to 95%.

The patient was treated with ACTH, receiving four brief courses over a three month period. He deteriorated progressively, but with each period of ACTH administration showed an abrupt drop in percentage of myeloblasts, and improved sense of well-being, increased appetite, and disappearance of petechiae, which reappeared during intervals between ACTH effect. Prior to the second and third courses of ACTH the patient appeared moribund, with generalized bleeding, jaundice, high fever and prostration. Prompt improvement occurred with each course of ACTH.

The close parallelism between the enzyme inhibitor patterns and neoplastic activity, as indicated by numbers of circulating immature cells, is illustrated in Figure 3. As in Case 1, the enzyme inhibitor responses consistently preceded changes in the cell count of the peripheral blood, but in this more acute form of the disease the lag was only 24 to 48 hours. By using just sufficient ACTH to bring

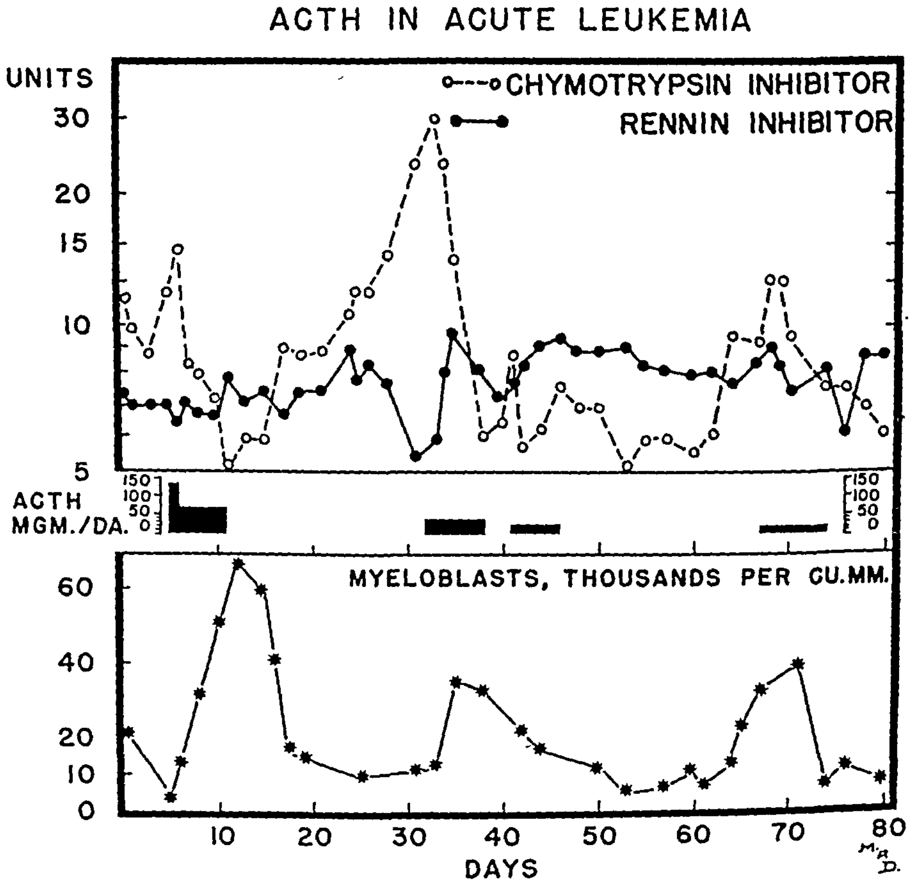

FIG. 3. RAPID REVERSAL OF THE ENZYME INHIBITOR PATTERN AND SUBSEQUENT HEMATOLOGIC IMPROVEMENT WITH ACTH THERAPY IN ACUTE LEUKEMIA

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[4] ACTH (Adrenocorticotropin), Lot No. 11302, Armour Laboratories.

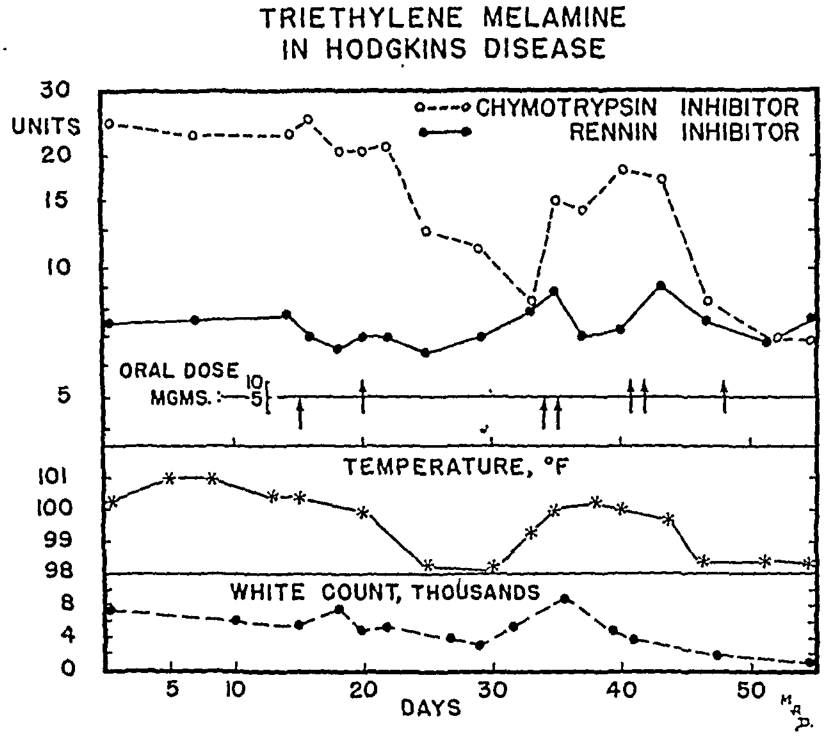

Fig. 4. Changes in Enzyme Inhibitor Balance Paralleling Clinical Response of Hodgkin's Disease to Triethylene Melamine Therapy

the enzyme pattern under relative control, repeated incomplete remissions were obtained in this patient.

Two other adults with acute leukemia, who were treated with ACTH for longer periods, also derived striking temporary clinical remissions accompanied by an abrupt fall in serum chymotrypsin inhibitor and rise in rennin inhibitor. However, second courses of ACTH were clinically and enzymatically ineffective in these cases.

### *Triethylene melamine in Hodgkin's disease*

On the assumption that the anti-mitotic action of the nitrogen mustards depends on the highly reactive ethylene portion of the molecule, a number of synthetic substances have been prepared containing several ethylene groups. One of these compounds, 2,4,6-tris (1-aziridyl)-s-triazine, was shown by Sugiura and Stock (10) to exert an inhibitory effect on certain animal tumors. This drug has been made available for clinical investigation under the name triethylene melamine.[5] It appears to possess definite value in palliative treatment of the lymphomas and, as indicated by the example below, its tumor inhibiting action traced by enzyme pattern is generally more rapid but less sustained than that previously reported for nitrogen mustard (4).

### *Case No. 4 (Figure 4)*

The first manifestation of Hodgkin's disease in this 59-year-old white male was limited to right cervical adenopathy which had been treated by surgical resection and x-ray. Three years later the patient re-entered the hospital complaining of severe constipation, anorexia, nausea with occasional vomiting, and weight loss of 15 pounds. In the few weeks preceding entry he had had fever daily to 101° with asthenia and malaise.

Physical examination showed a well-preserved 59-year-old white male, not obviously ill. Examination of the cervical area showed several old healed scars over the right sternocleidomastoid, in a region of thickening, induration, and pigmentation of the skin. There was no other adenopathy, and the spleen was not palpably enlarged. X-rays of the chest and gastrointestinal tract were normal. The white blood cell count was 7,300 with 84% neutrophiles, 12% lymphocytes, 3% monocytes, 1% eosinophiles. Red blood cell count 3,540,000, hemoglobin 10.8 gms.

The patient was treated with triethylene melamine as indicated in Figure 4. Response was rapid, with disappearance of fever and all gastrointestinal symptoms, reappearance of normal bowel movements, and disappearance of the right cervical nodes. A return of fever, nausea, and constipation within 10 days required repeated dosage of melamine. The second response was equally satisfactory, but severe leukopenia and anemia forced discontinuation of melamine in spite of the patient's statement that he felt "better than he had for six months," and was a "well man." He was subsequently treated with nitrogen mustard with prolonged clinical and enzymatic remission.

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[5] Triethylene melamine kindly furnished by Dr. D. A. Karnofsky, Lederle Laboratories Division, American Cyanamid Co.

As illustrated in Figure 4, treatment with 10 mg. oral doses of triethylene melamine resulted in prompt reduction in serum chymotrypsin inhibitor levels, although 5 mg. doses were relatively ineffective in this respect. Rennin inhibitor was not appreciably increased so that rather intensive therapy was required to restore the normal balance between the two factors. The periods of maximum enzyme response were associated with disappearance of fever and development of leukopenia. In this case the enzyme determinations were actually the only objective means of planning an adequate treatment schedule, although the patient's feelings of well-being were parallel to the periods of depression in the chymotrypsin inhibitor levels.

It is of interest that two months preceding the charted period, samples of this patient's serum had demonstrated an enzyme pattern compatible with neoplastic activity, but treatment was postponed because of lack of other objective evidences of disease. Subsequent events proved this to be another instance of the unique usefulness of serum enzyme inhibitor determinations for detection of tumor reactivation when other clinical confirmation is difficult to obtain.

Similar results were obtained with melamine therapy in another case of Hodgkin's disease, reticulum cell sarcoma, and chronic myelogenous leukemia. Although both clinical and enzymatic responses were observed, they were of short duration and severe marrow depression prevented frequent repetition of effective doses.

## INEFFECTIVE CHEMOTHERAPY

### *Cortisone in osteogenic sarcoma*

In view of the reported inhibitory action of cortisone on mouse osteogenic sarcoma (11) and mouse rhabdomyosarcoma (12) this steroid was given for a trial period to a patient with rapidly growing metastatic osteogenic sarcoma. In addition, since enzymatic responses to cortisone in lymphosarcoma are usually definite and rapid it was considered important to make comparable observations in another sarcoma which is generally resistant to treatment.

### *Case No. 5 (Figure 5)*

A 35-year-old white male was found to have osteogenic sarcoma of the right humerus and was treated with radical scapulo-thoracic amputation in February 1948. He remained clinically well until August 1949 when he developed a metastasis to the left seventh rib. This local metastasis was surgically removed for relief of pain. The patient then developed a slowly enlarging tumor mass at the site of surgery, and in May 1950 had rapid development of extensive pulmonary and abdominal metastases. This sudden increase in disease activity was associated with a soaring rise in chymotrypsin inhibitor levels and diminishing rennin inhibitor titers.

On this admission, the patient was ambulatory, showed recent weight loss, the previously mentioned surgical deformities, a hard fusiform mass 15 × 5 × 5 cm. along the lateral aspect of the left seventh rib, and multiple stony-hard, grapefruit-sized masses in the abdomen, with considerable ascites. X-rays showed multiple calcified pulmonary metastases, and diffuse calcification throughout the abdomen, corresponding to the tumor masses.

Repeated laboratory work was not contributory to the present discussion, multiple blood counts, serum calcium,

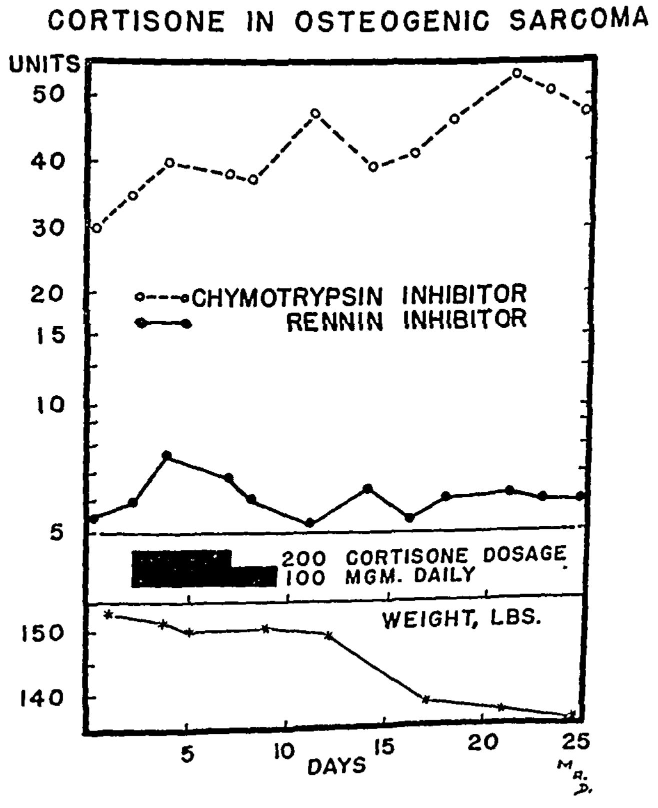

FIG. 5. ABSENCE OF ENZYME INHIBITOR CHANGES IN OSTEOGENIC SARCOMA RESISTANT TO CORTISONE

phosphorous, alkaline and acid phosphatases, and liver function tests being essentially within the normal ranges.

He received cortisone 1,200 mg. in eight days, with no observable clinical or enzymatic response. During the period of treatment the patient declined progressively and the tumor masses enlarged visibly.

This case illustrates the value of the two serum enzyme inhibitor determinations in confirming complete lack of response when a drug is used in a frankly investigational manner. The sensitivity of these proteolytic enzyme inhibitors to effective chemotherapy is such that an eight day trial period has been found ample for the evaluation of most drugs. Had there been even slight enzymatic evidence of decreased tumor activity during this period, further treatment with the hormone might have been considered in an attempt to achieve clinically detectable improvement.

### *Progesterone in carcinoma of the prostate*

After a period of palliation by either orchiectomy, estrogen therapy or a combination of the two, most patients with carcinoma of the prostate finally become resistant to this type of treatment. It is of interest in such cases, therefore, to attempt to restrain the disease further by again altering the hormone balance. Clinical responses to progesterone and testosterone have been variable and individualized, and the sensitivity of these serum inhibitor substances has been of considerable assistance in our hands, in measuring the effects objectively and avoiding continued treatment with tumor stimulating or ineffective hormones.

#### *Case No. 6 (Figure 6)*

This 64-year-old white male with carcinoma of the prostate had had treatment for two and one-half years, consisting successively of radical prostatectomy, orchiectomy, estrogen administration and palliative deep x-ray therapy to multiple areas of osteoblastic metastases of the pelvis, femora, vertebrae, and ribs. For six months preceding the current admission he developed progressively severe pain in the involved areas of bony metastases, requiring large doses of narcotics for relief.

Physical examination showed only pallor, evidence of weight loss, and surgically absent prostate and testicles, although the patient was still well-nourished and ambulatory on crutches. X-rays confirmed the extensive bony metastases. Serum acid phosphatase was 7.8 Bodansky units (normal 2 to 9 units). The patient had a moderate hypochromic anemia corrected by whole blood transfusions.

Because he had previously responded well to changes in hormone balance to which he was now refractory, it was thought worth while to try another of the steroid hormones. Accordingly, with the unfavorable enzymatic pattern shown in the graph, he was given progesterone 100 mg. intramuscularly daily for 19 days. He received no benefit, his pain was unaffected, and he continued to lose weight.

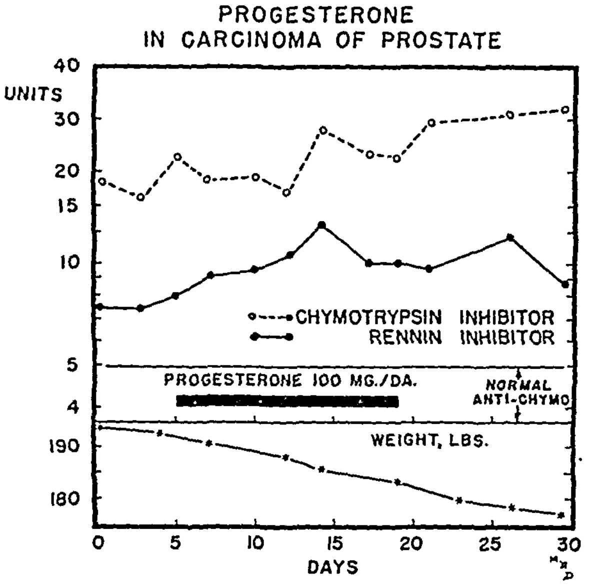

FIG. 6. INEFFECTIVE TREATMENT OF PROSTATIC CARCINOMA WITH PROGESTERONE AND CORRESPONDING LACK OF SIGNIFICANT ENZYME INHIBITOR RESPONSE

In this case, previous serial enzyme inhibitor determinations following estrogen and testosterone administration and withdrawal had given excellent correlation with clinical status, and showed great sensitivity in response to these hormones. Therefore, since progesterone did not alter the enzyme balance, it was concluded that the drug was ineffective in this patient. Without such data, treatment might have been continued needlessly for a longer period, since clinical response in advanced metastatic prostatic carcinoma is frequently delayed, and early x-ray changes are not expected. Serial acid phosphatase determinations were not helpful in this patient since previously they had remained within normal limits in spite of clinical exacerbations and remissions.

### *Testosterone in carcinoma of the prostate*

#### *Case No. 7 (Figure 7)*

This 63-year-old white male was admitted to the hospital with complaints of increasing pain in the pelvis, low back, and posterior thighs for the preceding several weeks.

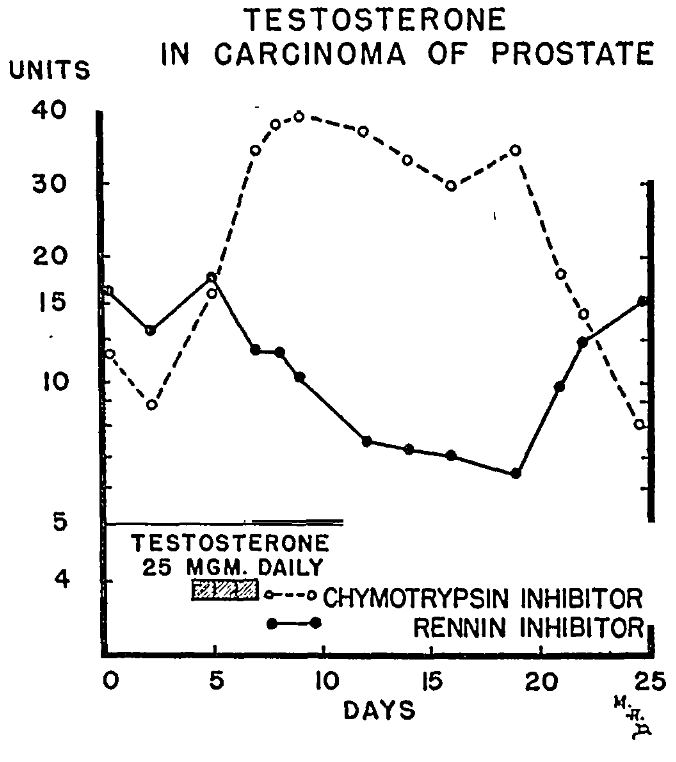

FIG. 7. STIMULATION OF PROSTATIC CARCINOMA BY TESTOSTERONE ACCOMPANIED BY IMMEDIATE ENZYMATIC EVIDENCE OF INCREASED TUMOR ACTIVITY

He had previously been followed in the out-patient department of the tumor service for 11 months following original diagnosis of adenocarcinoma of the prostate established by transurethral resection, following which bilateral orchiectomy was performed. The patient then felt quite well for five months, at which time he was started on ethinyl estradiol because of back, pelvic and thigh pain, due to extensive bony metastases. This relieved him for another five months, but he again developed increasingly severe pain which occasioned this present admission.

Physical examination on entry showed a well-developed, slightly obese 63-year-old white male complaining of moderately severe pelvic and back pain, and able to walk only a few steps with great difficulty. The testicles were surgically absent, and prostatic examination revealed scattered small stony-hard nodules in both lateral lobes. X-rays showed extensive osteoblastic metastatic nodules involving the lumbar spine, sacrum, bony pelvis, and upper ends of both femora.

Soon after admission estrogen medication was discontinued, and the patient improved slightly, both clinically as manifested by a decrease in pain, and as measured by the enzyme inhibitor determinations. However, after a month of this observation, he again began to have increased pain in the areas of bony metastases. It was then decided to try testosterone because of previously experienced paradoxical palliative effects of male steroid administration in cases of prostatic carcinoma resistant to estrogens. Accordingly, the patient received testosterone propionate, 25 mg. intramuscularly daily for three doses. Within 24 hours of the first injection, his pain was acutely intensified, and he was hardly able to move about in bed. Coincidentally there was an immediate effect on the enzyme pattern, with marked elevation of the chymotrypsin and fall in the rennin inhibitor levels. Although five doses of 25 mg. each had been tentatively planned, the testosterone was stopped after the third dose because of the alarming change in the enzyme inhibitor pattern. On the fourth day, the patient was mentally confused, appeared prostrate, flushed, apprehensive, and required generous use of narcotics for pain relief. He gradually improved, and three weeks after stopping the testosterone was almost free of pain, fully ambulatory, and was discharged from the hospital. At the time of his discharge, his enzyme pattern showed declining chymotrypsin, and rising rennin inhibitor levels.

This case illustrates several interesting points. Figure 7 shows clearly the acute disturbance of enzyme inhibitor pattern immediately after testosterone administration. Also shown is the return of these inhibitors to pre-treatment levels following cessation of the androgen. The clinical parallelism with these determinations was disconcertingly convincing. Of further interest is the fact that the patient improved enough to be discharged from the hospital following recovery from this period of acute androgen-induced exacerbation. Although several other estrogen-resistant cases of prostatic carcinoma with previous orchiectomy have shown a similar sensitivity to small doses of testosterone, the same response has not been obtained in all such patients. In no other malignancies, except carcinoma of the breast and prostate, has any change in chymotrypsin and rennin inhibitor concentrations been observed following the administration of androgens.

### *Guanazolo in acute leukemia*

Considerable interest has been created by the work of Kidder and associates (13, 14) in the possibility of selectively blocking nucleoprotein synthesis in neoplastic tissues by the administration of unnatural purines. Guanazolo[6] (8-azaguanine), a synthetic guanine antagonist, successfully inhibited the progress of some carcinomas and leukemias in mice. Unfortunately, similar results have not been reported in human malignant disease. An example of the clinical trial of guanazolo therapy is presented in which even minor changes in serum enzyme inhibitor balance failed to appear and the course of the disease was unaltered.

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[6] Guanazolo, Lot No. 7-9710, kindly furnished by Dr. D. A. Karnofsky, Lederle Laboratories Division, American Cyanamid Co.

### *Case No. 8 (Figure 8)*

A 40-year-old white male was transferred to this hospital with the previously established diagnosis of acute leukemia. Complaints of about six weeks duration included marked weakness, fatigue, fever, and purpura of forearms, calves, and thighs.

Physical examination on admission showed a tired-looking, weak, sallow-complexioned white male appearing acutely and chronically ill. Abnormal findings included swelling of the soft tissues bilaterally in the submaxillary area, hyperplasia and friability of the gums with areas of ulceration, several small areas of absorbing blood pigment over the calves and forearms. There was no peripheral adenopathy nor palpably enlarged spleen. Laboratory findings on entry included white blood cell count of 90,000, with differential count of 95% neutrophiles (including one stab cell, three promyelocytes, and 83 blasts), a red blood cell count of 2,460,000, hemoglobin 9.6 gms. Platelets 110,000.

The patient pursued a progressively downhill course of three months duration. He was treated with the usual supportive methods including multiple whole blood transfusions, and antibiotics as needed. He was tried successively on several drugs of the "metabolic blocking agent" classification, including guanazolo, of which he received 600 mg. orally daily for 14 days. There was no noticeable clinical improvement during this period.

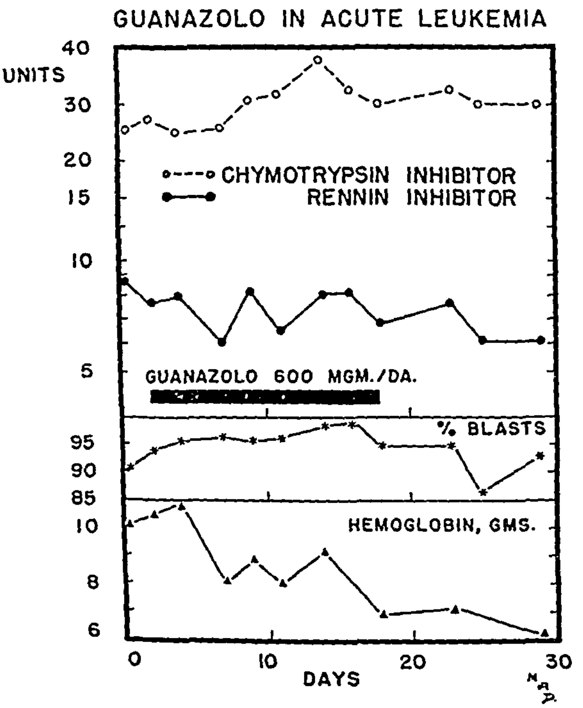

FIG. 8. FAILURE OF GUANAZOLO TO INDUCE REMISSION IN ACUTE LEUKEMIA OR TO ALTER ENZYME INHIBITOR CONCENTRATIONS

Figure 8 illustrates complete absence of serum enzymatic inhibitor response to guanazolo therapy. Clinically, the patient continued to deteriorate while the percentage of blasts in the peripheral blood remained between 85 and 90%. The red blood cell count and hemoglobin declined steadily during this period, in spite of daily transfusions. In this instance, it is apparent that guanazolo, which was of therapeutic value in experimental leukemia, did not exert a similar effect in the comparable disease in man.

## DISCUSSION

The sensitivity of chymotrypsin and rennin inhibitors of the serum to changes in neoplastic activity is of theoretical as well as practical interest. The mechanism by which rapid alteration in the balance between the two factors is brought about in response to effective chemotherapy is not known. As illustrated by the cases presented in this paper, there is no direct action of the medication on these enzyme systems. Only if the drug exerts a stimulating or restraining effect on the tumor itself will the enzyme inhibitor concentrations change. Serial determinations of these enzyme inhibitors appear therefore to yield information by which the course of the malignant process may be charted. The value of such data in the clinical investigation of palliative drugs and in management of the cancer patient has been substantiated by daily use of the method for three years, during which time over 30,000 determinations have been made.

Of necessity, in order to demonstrate the parallelism between the action of a drug on the tumor and changes in the enzyme inhibitor pattern, cases had to be selected in which the effects of treatment were clinically obvious. In by far the majority of cases encountered in a tumor service, however, the enzyme results constitute the only simple and objective indicator of the patient's progress. Frequently an individual with cancer, in his enthusiasm over a new drug, may feel that he is benefited but the enzyme patterns reveal whether or not there is any basis for his subjective improvement. On the other hand, highly significant but weak, sub-clinical effects on the tumor may be produced by an agent under investigation which would have escaped notice without a sensitive measure of neoplastic activity. Likewise, reactivation of tumor growth after a period of remission may often be detected enzymatically long before symptoms appear, thereby making it possible to give further palliative treatment earlier and more effectively.

In the interpretation of enzyme inhibitor determinations, it should be emphasized that the ratio between the two factors is as important as their individual absolute values. This point has been discussed in detail by West, Rapaport, and Tempereau (4) in describing the relationship between the enzyme inhibitors and clinical response to nitrogen mustard therapy. The aim of treatment is to produce and maintain the serum chymotrypsin inhibitor at five units or less, and rennin inhibitor at 15 units or more, since it has been found that this equilibrium between the two factors is uniformly associated with complete tumor inhibition or regression. Usually the higher the rennin inhibitor titer, the more complete and prolonged a remission is likely to be. Patients with known residual disease which has remained inactive for many years, show in addition to normal serum chymotrypsin inhibitor, the highest rennin inhibitor levels of all, at times reaching 100 units. Observations to date have suggested that as a working hypothesis the chymotrypsin inhibitor might be considered an index of "neoplastic activity" and the rennin inhibitor in some unexplained manner a reflection of "host resistance." Experiments are in progress to determine the source of these enzyme inhibitors, their biochemical nature, and the mechanism of their release into the circulation.

## SUMMARY

1. Evidence is presented which indicates that the equilibrium between chymotrypsin and rennin inhibitors of the serum may be utilized as a sensitive measure of the effectiveness of palliative therapy in the neoplastic diseases.

2. Several examples of effective chemotherapy are described in which, regardless of the drug used or the type of the malignancy, inhibition of tumor growth is reflected by similar changes in the concentration of serum enzyme inhibitors.

3. Ineffective chemotherapy does not alter the enzyme inhibitor pattern while tumor stimulating agents produce enzymatic changes associated with increased neoplastic activity.

4. The sensitivity, consistency and close correlation of these two factors with the clinical status of the cancer patient suggest that the method possesses considerable practical, as well as research value in assisting the charting of the course of neoplastic disease.

## ACKNOWLEDGMENTS

The authors are greatly indebted to Willard F. Keye and Bronnetta L. Scott for technical assistance, and to Mary A. Dubbin for preparation of the figures.

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*v*-triazolo (*d*) pyrimidine on carcinoma, sarcoma, osteogenic sarcoma, lymphosarcoma, and melanoma in animals. Cancer Research, 1950, 10, 244.

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# ESTIMATION OF TOTAL BODY SODIUM BY ISOTOPIC DILUTION. I. STUDIES ON YOUNG ADULTS[1, 2, 3]

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(Submitted for publication December 13, 1950; accepted, March 19, 1951)

Although the amount of sodium contained within the human body has been determined in a number of fetuses and newborns by direct chemical analysis, no such data are available for the adult. This value has been estimated (by means which are not clear) to be 63 Gm. by Shohl (2), 56 Gm. by Hackh (3), and 105 Gm. by Sherman (4) for a 70 Kg. man. The literature contains reports of the chemical analysis of one adult carcass, but unfortunately values for sodium are not given (5). The importance of sodium in the electrolyte economy of the human body prompted us to undertake a study of the feasibility of determining total body sodium by the isotopic dilution method.

*In vitro* applications of isotopic dilution analysis are well known, and present no particular difficulties under conditions wherein the system to be studied is a closed one. The method was applied to man for the determination of total body water in 1934 by Hevesy and Hofer (6) who used deuterium, a stable isotope, in their experiments. More recently radioactive hydrogen has been used for the same purpose (7). Although Kaltreider and his co-workers (8) made the suggestion several years ago that they were measuring total body sodium in experiments aimed at determination of extracellular fluid volume, F. D. Moore (9) was the first to emphasize the potentialities of the isotopic dilution method for the measurement of total body sodium and potassium in addition to total body water in man, and reported a value of 59 meq. of sodium per Kg. in an adult male weighing 66 Kg.

This paper reports the result of a study of the measurement of total body sodium (perhaps a more accurate term would be "total exchangeable sodium") in a series of healthy young adults. In brief, the procedure employed was to introduce a known amount of radioactive sodium into the body, allow it to become distributed through the body tissues, and then determine the serum sodium specific activity.[5] If allowance is made for excretion of radiosodium during the equilibration period, the total amount of sodium in equilibration with radiosodium can then be calculated. It should be pointed out that this method makes no distinction between extracellular and intracellular sodium.

## METHODS

Radioactive sodium ($Na^{24}$, half-life 15 hours) was manufactured in the Washington University cyclotron and prepared in our laboratory as a sterile isotonic solution of sodium chloride. Accurately measured volumes were given intravenously according to the method described by Barnett and Fellers (10). The total volume of isotonic saline injected, including the "wash" saline, did not exceed 5 cc., corresponding to 0.8 meq. of sodium, an amount negligible in relation to the total body content of sodium. The amount of $Na^{24}$ used in these experiments varied from 1 to 1½ microcuries per Kg. of body weight; this quantity has been calculated to deliver a total radiation dose of from 0.11 to 0.17 roentgen equivalent physical (11). Samples of venous blood were obtained from a vein other than that used for injection of radiosodium; serum was separated from the cells within an hour.

Members of the resident house staff and medical students served as subjects for these experiments. They were all in good health and ranged in age from 20 to 34 years. They were allowed normal activity and consumed their usual diet during the experiment, but were instructed to avoid addition of excess salt to their food and to refrain from excessive physical exercise. Samples of normal bone and brain tissue and cerebrospinal fluid were obtained from patients at the time of regularly scheduled operations.

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[1] This work was carried out under grants from the Children's Research Foundation and the U. S. Atomic Energy Commission.

[2] The cooperation of the Departments of Neurosurgery and Orthopedic Surgery of the Washington University School of Medicine and of the Cyclotron Group at Washington University is gratefully acknowledged.

[3] A preliminary report of these studies has been published elsewhere (1).

[4] Present address: Southwestern Medical School, Dallas, Texas.

[5] Radioactivity per unit weight of sodium.

One cc. amounts of serum, urine, and cerebrospinal fluid, in duplicate, were pipetted into metal planchets, 33 mm. in diameter, and dried at 70° C. after the addition of one drop of synthetic detergent (Acidolate). Aliquots of the injected solution were similarly prepared in triplicate. The samples were counted with an end-window Geiger tube attached to an autoscaler. Counting periods in most instances were of such length that statistical variations due to random fluctuations of radioactive emission were ± 1.6% (standard deviation) or less for the serum and standard samples, and ± 3% or less for urine samples.[6] Standard solutions of $Na^{24}$ were assayed at frequent intervals during each counting run, and a decay curve constructed for each series of determinations; from this curve appropriate decay corrections were made. Coincidence and background effects were also accounted for, and the counter efficiency was checked periodically with a radium D-E standard.

Bone and brain samples were obtained in the operating room, care being taken to avoid contamination with saline sponges, blotted on filter paper to remove excess blood, and put immediately in tared stoppered bottles. The tissues were weighed and then digested in nitric acid until the resulting solution was clear; aliquots of this solution were then prepared for counting in one of two ways. In our earlier experiments the solution was transferred to porcelain capsules, dried on an electric hot plate and weighed just prior to counting. Corrections for self-absorption could then be made by reference to a self-absorption curve constructed from assays of beef bone to which known amounts of $Na^{24}$ had been added. These were prepared in the same manner as the human bone samples and ranged in weight (as plated for counting) from 13 to 292 mg. per square centimeter. In the case of brain tissue self-absorption corrections were not necessary. Samples of serum for comparison with bone and brain were also plated in porcelain capsules to equalize back-scattering effects. Later in the course of the study counting was done with a thin walled dipping counter, the samples of bone digest and of serum being assayed in liquid form so that self-absorption corrections were no longer necessary.

Analyses of serum, cerebrospinal fluid, urine, and bone for sodium were carried out by the zinc uranyl acetate gravimetric method (12). Dry, rather than wet ashing was used for the serum. Aliquots of the nitric acid bone digest were evaporated to dryness and ashed in a muffle furnace at 540° C. The ash was then dissolved in nitric acid, an excess of ammonia added to remove the greater part of the calcium and phosphate, and an aliquot of the filtrate evaporated to dryness. The residue was taken up in water and quantitatively transferred to the zinc uranyl acetate reagent in the usual manner. Analyses of the brain tissue were made by the flame photometer.

For the purpose of chloride analysis, the preliminary nitric acid digestion of bone was made in the presence of silver nitrate, which has been shown, in our laboratory, not to interfere with the gravimetric determination of sodium. Chloride was determined in serum and bone digest by the modified Volhard-Harvey titration method (13).

Calculations were made as follows: serum was obtained from the subjects at times ranging from 18 to 24 hours (in two, a 17 hour period was used) following the intravenous administration of radiosodium, and urine was collected over the same period of time. Assuming complete distribution of the injected radiosodium, serum specific activity should equal total body specific activity, so that

$$\text{total exchangeable sodium (TES)} = \frac{Na^{24}\text{ injected} - Na^{24}\text{ excreted}}{\text{serum } Na^{24}/\text{serum sodium}}.$$

No corrections were made for the serum water content, the Donnan effect, or the possibility of slight differences in mobility between $Na^{24}$ and $Na^{23}$ ions.

## RESULTS

The results of 36 determinations of total exchangeable sodium (TES) in 32 healthy young adults are set forth in Table I. The average value for males, 41.9 meq. per Kg. of body weight, is surprisingly close to Shohl's estimate of 39 meq. per Kg. (2). The range of values is so great that one hesitates to speak of an average value for the average adult male. The spread of values (32.3 to 54.1 meq.), which would appear to be too great to be explained solely on experimental error, tends to remain (but to a somewhat less degree) when the calculations are made on the basis of height or surface area. Two separate determinations of TES were made in each of four subjects, but since the intervals between determinations were four to 22 months, they cannot be used to assess experimental errors. A considerable proportion of the body sodium is in skeleton and muscle; since the amount of these two tissues probably varies markedly among individuals of the same weight, the wide range of values noted in Table I might be expected.

The average value for females (39.5 meq. per Kg.) is slightly lower than that for males and this difference persists when the calculations are made on the basis of height and surface area; however, the magnitude of the standard deviations would indicate that the average values for the two groups are not significantly different.

These values represent the amount of sodium within the body which comes into equilibrium with administered radiosodium during the ex-

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[6] In a few instances counting rates were so low that errors in estimating serum radioactivity were ± 3%.

TABLE IA

*Total exchangeable sodium in adult males*

In the subjects indicated by the asterisk two samples of blood, drawn at the times indicated, were analyzed for specific activity and the results averaged.

| Subject | Age | Weight | Height | Surface area | Equilibration period | TES | Meq. Na per Kg. | Meq. Na per cm. | Meq. Na per $m^2$ |
|---|---|---|---|---|---|---|---|---|---|
| | *years* | *Kg.* | *cm.* | $m^2$ | *hrs.* | *meq.* | | | |
| *E. C. | 23 | 100 | 193 | 2.30 | 23 | 3230 | 32.3 | 16.7 | 1405 |
| *A. H. | 28 | 91.6 | 193 | 2.22 | 22½ | 3955 | 43.2 | 20.5 | 1780 |
| *P. J. | 24 | 84.8 | 180 | 2.04 | 22½ | 2753 | 32.5 | 15.3 | 1350 |
| M. S. | 25 | 83.8 | 178 | 2.00 | 19½ | 3125 | 37.2 | 17.6 | 1562 |
| J. F. | 29 | 79.8 | 170 | 1.91 | 19½ | 2805 | 35.1 | 16.5 | 1470 |
| F. C. | 33 | 79.3 | 182 | 1.98 | 17 | 2815 | 35.5 | 15.4 | 1420 |
| A. R. | 26 | 77.6 | 177 | 1.94 | 23 | 3195 | 41.2 | 18.0 | 1645 |
| J. R. | 29 | 76.9 | 177 | 1.93 | 24 | 3315 | 43.1 | 18.7 | 1715 |
| W. B. | 27 | 76.2 | 183 | 1.97 | 19½ | 3210 | 42.1 | 17.5 | 1630 |
| R. M.$^1$ | 23 | 75.6 | 172 | 1.88 | 20 | 2987 | 39.5 | 17.4 | 1590 |
| *R. M.$^2$ | 25 | 75.4 | 172 | 1.88 | 18–19½ | 3051 | 40.4 | 17.7 | 1623 |
| V. T. | 34 | 75.4 | 171 | 1.87 | 24 | 3180 | 42.2 | 18.6 | 1700 |
| R. G. | 24 | 75.4 | 188 | 2.00 | 22½ | 3785 | 50.2 | 20.1 | 1895 |
| L. K. | 27 | 74.4 | 178 | 1.91 | 22½ | 2907 | 39.1 | 16.3 | 1520 |
| S. S.$^1$ | 25 | 74.1 | 183 | 1.95 | 23 | 3700 | 49.9 | 20.2 | 1898 |
| S. S.$^2$ | 25 | 75.2 | 183 | 1.96 | 17½ | 3525 | 46.9 | 19.3 | 1800 |
| C. C. | 24 | 74.1 | 180 | 1.93 | 19½ | 3060 | 41.3 | 17.0 | 1585 |
| *R. B. | 28 | 73.9 | 190 | 1.99 | 22½ | 3445 | 46.6 | 18.1 | 1730 |
| J. M. | 24 | 73.9 | 180 | 1.92 | 24 | 3515 | 47.6 | 19.5 | 1830 |
| *R. B. | 24 | 73.5 | 176 | 1.99 | 23 | 2560 | 34.8 | 14.6 | 1355 |
| *R. H. | 27 | 72.9 | 166 | 1.80 | 18–24 | 2978 | 40.8 | 17.9 | 1653 |
| *M. C. | 23 | 71.9 | 175 | 1.86 | 17–22½ | 3075 | 42.8 | 17.6 | 1650 |
| *B. H. | 25 | 71.8 | 182 | 1.91 | 22½ | 2440 | 34.0 | 13.4 | 1280 |
| *J. C. | 29 | 70.2 | 168 | 1.79 | 18–22 | 2748 | 39.1 | 16.4 | 1535 |
| *W. G.$^1$ | 24 | 69.1 | 190 | 1.94 | 18–21½ | 3263 | 47.2 | 17.2 | 1680 |
| *W. G.$^2$ | 25 | 68.1 | 189 | 1.92 | 18½ | 3685 | 54.1 | 19.5 | 1930 |
| *E. L. | 24 | 69.8 | 178 | 1.86 | 18–23½ | 3300 | 47.3 | 18.6 | 1775 |
| *H. M.$^1$ | 29 | 58.3 | 164 | 1.62 | 22 | 2753 | 47.2 | 16.8 | 1698 |
| H. M.$^2$ | 29 | 60.6 | 164 | 1.65 | 20 | 2487 | 41.1 | 15.2 | 1518 |
| Average | | | | | | | 41.9 | 17.5 | 1628 |
| Standard deviation | | | | | | | 5.6 | 1.46 | 170 |
| Coefficient of variation | | | | | | | 13.4% | 8.3% | 10.5% |

perimental period. The relation of this value, "total exchangeable sodium," to the actual quantity of sodium in the body will be discussed below.

In two subjects (not included in Table I) radiosodium was administered orally rather than intravenously. The values for total body sodium in these subjects were 29.9 and 23.7 meq. per Kg., respectively, and were so much lower than those

TABLE IB

*Total exchangeable sodium in adult females*

In the subjects indicated by the asterisk two samples of blood, drawn at the times indicated, were analyzed for specific activity and the results averaged.

| Subject | Age | Weight | Height | Surface area | Equilibration period | TES | Meq. Na per Kg. | Meq. Na per cm. | Meq. Na per $m^2$ |
|---|---|---|---|---|---|---|---|---|---|
| | *years* | *Kg.* | *cm.* | $m^2$ | *hrs.* | *meq.* | | | |
| *R. C. | 26 | 81.6 | 171 | 1.93 | 18–22 | 2913 | 35.7 | 17.0 | 1509 |
| *D. B. | 20 | 62.6 | 169 | 1.71 | 18–20 | 2549 | 40.7 | 15.1 | 1490 |
| H. W. | 27 | 61.3 | 165 | 1.66 | 18 | 2255 | 36.8 | 13.7 | 1358 |
| *M. K. | 24 | 57.7 | 162 | 1.60 | 18–20 | 2295 | 39.8 | 14.2 | 1432 |
| F. D. | 25 | 55.9 | 163 | 1.59 | 18 | 2265 | 40.6 | 13.9 | 1427 |
| H. H. | 24 | 54.4 | 164 | 1.58 | 23 | 2335 | 41.1 | 13.6 | 1413 |
| M. G. | 27 | 51.8 | 162 | 1.53 | 18 | 2153 | 41.6 | 13.3 | 1405 |
| Average | | | | | | | 39.5 | 14.4 | 1433 |
| Standard deviation | | | | | | | 2.29 | 1.28 | 51.4 |
| Coefficient of variation | | | | | | | 5.8% | 8.9% | 3.6 % |

found in any of the subjects given radiosodium intravenously that attempts to use the oral route of administration were quickly abandoned.

## DISCUSSION

*Assumptions.* For the purpose of these experiments several assumptions had to be made. First, our subjects were considered to be in sodium balance. For that reason, they were encouraged to engage in normal activity and to eat their usual diet. Experiments were not done during the hot weather. Since radiosodium equilibration would be disturbed by states of either positive or negative sodium balance, every effort was made to avoid change in living habits during the experiment. The prerequisite of a relatively stable sodium economy would appear to preclude the use of this method in the study of some disease states, and would suggest that errors might be introduced by requiring the subjects to fast during the experiment. Secondly, fecal and cutaneous losses of radiosodium were considered to be negligible. No studies of fecal or cutaneous excretion of radiosodium are available in the literature; however, the major pathway of sodium excretion is by way of the kidney. In children, Macy (14) found that only 2% of the total sodium excretion was by way of the gastrointestinal tract. In our experiments the quantity of radiosodium excreted in the urine during the equilibration period averaged 5.6% of the injected dose, with a range of 2.7 to 10.1%. Since fecal and cutaneous losses of radiosodium could hardly represent more than a small fraction of the urinary loss (assuming that Na$^{24}$ is handled by the body similarly to Na$^{23}$), only a small error is incurred in the final results by their omission. None of our subjects perspired profusely or had loose stools during the experimental period.

Thirdly, the sodium turnover during the equilibration period was considered to be small in relation to the total sodium content of the body. Measurements of urinary sodium excretion were made in 11 of our subjects, and ranged from 76.9 to 360 meq., or 2.7 to 12% (average 5.2%) of the calculated total body sodium.

Lastly, is the administered radiosodium in equilibrium with all of the body sodium? Information on this point is largely indirect since no direct chemical analyses of adults are available with which to compare our data.

*Change in apparent volume of distribution of Na$^{24}$ with time.* One means of attacking the problem of radiosodium mixing is through a study of the change in radiosodium "space" with time. Although Burch and co-workers (15) have made an exhaustive study of the curve of serum radioactivity at periods up to an hour and Kaltreider and co-workers (8) have extended the period of observation to 12 hours after radiosodium administration, data are lacking for later periods. Figure 1 presents our data in regard to this question. In it are plotted the total radiosodium spaces calculated as

$$\frac{\text{radioactivity injected} - \text{radioactivity excreted}}{\text{radioactivity/cc. serum}}$$

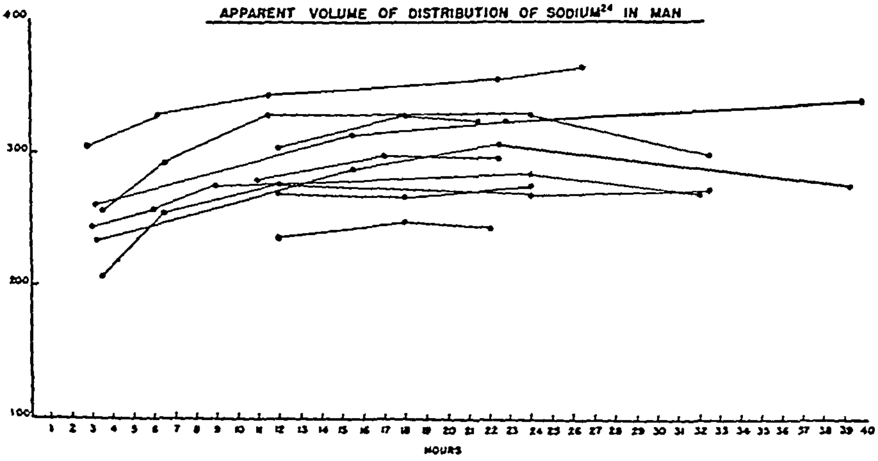

FIG. 1. CURVES OF APPARENT VOLUME OF NA$^{24}$ DISTRIBUTION, OR SODIUM "SPACE" (CC. PER KG.) IN 10 ADULT SUBJECTS ON A NORMAL DIET

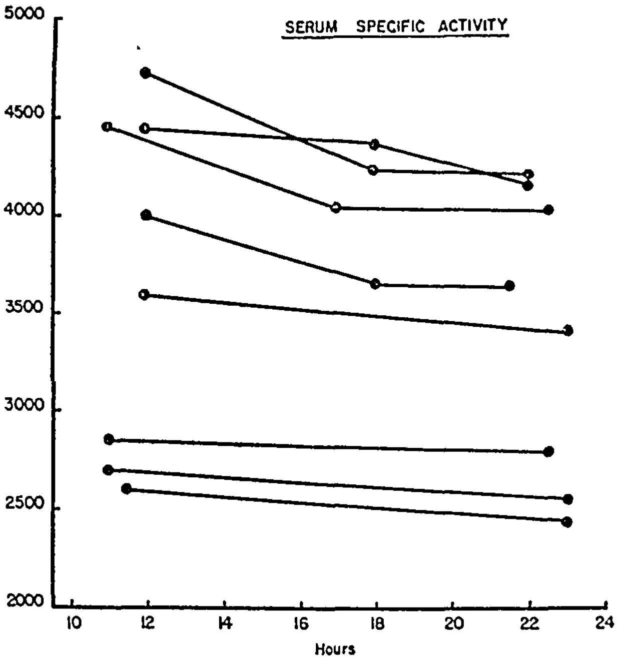

FIG. 2. CURVES OF SERUM SPECIFIC ACTIVITY, COUNTS PER MINUTE PER MEQ. NA, IN EIGHT ADULT SUBJECTS ON A NORMAL DIET

in 10 adults at times ranging from 2½ to 39½ hours after injection. The irregularities in the curves occurring after 30 hours are probably the result of errors due to extremely low counting rates. It is seen that by 18 hours the apparent volume of radiosodium distribution has become fairly stable. Additional exchange after this period occurs at an extremely slow rate. Some of the increase may possibly be due to extra-renal $Na^{24}$ losses not accounted for in our experiments.

In Figure 2 data on change of serum specific activity during the 11–23 hour period following radiosodium administration are presented for eight subjects. A definite leveling off of the curves is seen to occur by 18 hours.

One possible source of error in these experiments has to do with the fact that injected sodium undoubtedly mixes with dietary sodium taken during the course of the experiment (16). To minimize effects of dietary sodium, four additional subjects (not included in Table I) were studied on a low sodium diet. They took all of their food and fluids under the supervision of the dietitian at the St. Louis Children's Hospital, and consumed a diet adequate in every respect save sodium. The calculated amount of sodium in the food actually consumed varied from 8.52 to 15.9 meq. per day. After three days of the diet, radiosodium was given intravenously and the curve

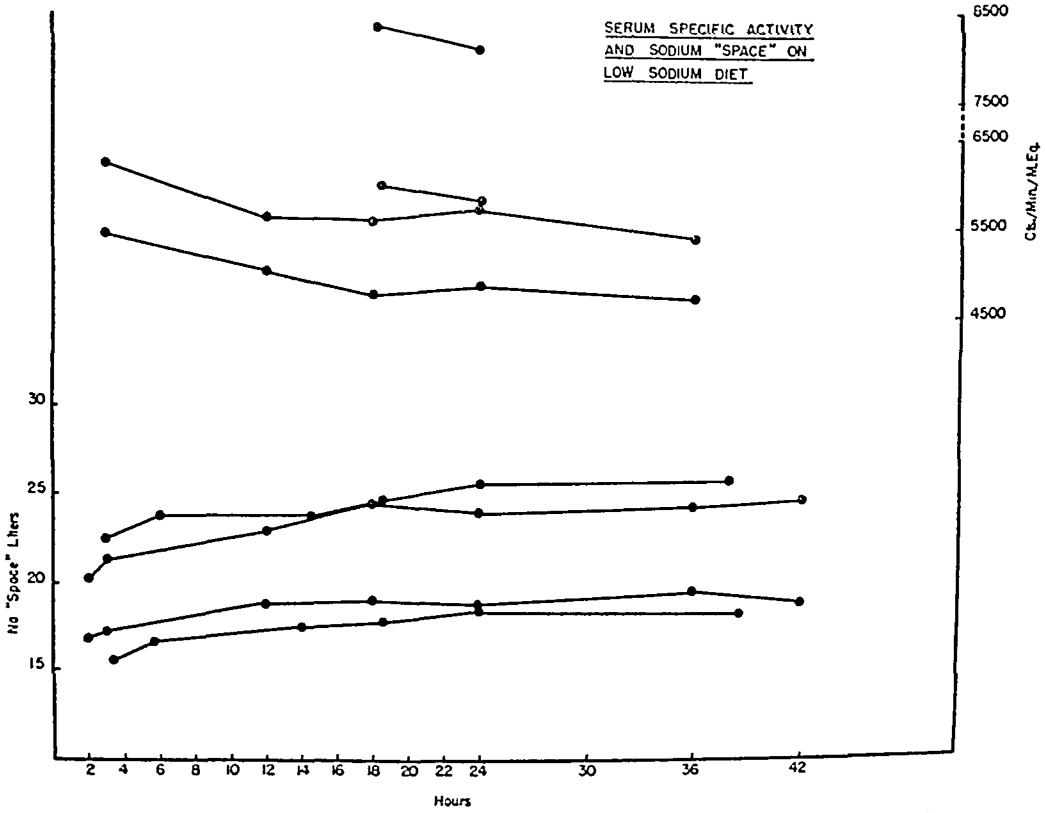

FIG. 3. CURVES OF $NA^{24}$ "SPACE" (LITERS) AND OF SERUM SPECIFIC ACTIVITY (COUNTS PER MINUTE PER MEQ. NA) IN FOUR SUBJECTS WHO WERE CONSUMING A LOW SODIUM DIET

of $Na^{24}$ equilibration determined in the usual way, the diet being continued for the duration of the study period. On such a regime urinary sodium excretion (after the first two days) ranged from 13.2 to 27.2 meq. daily and radiosodium excretion was greatly reduced, ranging from 0.5 to 0.7% of the administered dose in 24 hours. Thus a situation in which sodium turnover within the body was reduced to a minimum was produced. The curves of these data, presented in Figure 3, are quite similar to the previous ones: we may therefore conclude that dietary sodium in usual amounts does not appreciably disturb radiosodium equilibration. Larger amounts of radiosodium (approximately 2 microcuries per Kg.) were used in these experiments, so that the period of accurate observation could be prolonged.

It was on the basis of these observations that the 18–24 hour period was selected as being most suitable for total body sodium determinations.

*Tissue distribution of radiosodium.* Studies in animals have shown that there are at least two types of tissue present in the body, when a classification is made on the basis of the rapidity with which radiosodium mixes with tissue sodium. Certain tissues such as muscle, skin, kidney, and liver equilibrate very rapidly (within 20–60 minutes), whereas in others—bone, brain, and testis—mixing may not be complete for at least 12 hours after radiosodium administration (17). The data of Kaltreider and his associates (8) included studies of human pleural, ascitic and spinal fluid and indicated that nine to 12 hours were required for complete mixing in these compartments. However, since pleural and ascitic effusions represent abnormal fluid collections, normal extracellular fluid may well equilibrate at a different rate (probably more rapidly) than these figures would indicate. That abnormal subjects are unsuitable for tissue equilibration studies is

TABLE II

*Tissue specific activity*

| Subject | Age | Source | Time *hours* | Sodium content *mg. per Gm. wet tissue* | Ratio tissue/serum specific activity | Ratio tissue/serum chloride |
|---|---|---|---|---|---|---|
| | | | Bone | | | |
| Sa. | 37 yr. | lumbar lamina | 2 | 2.70 | 0.432 | |
| C. | 44 yr. | rib | 4 | 4.95 | 0.169 | 0.198 |
| H. | 76 yr. | skull | 6½ | 5.85 | 0.196 | |
| M. | 11 yr. | humerus cortical<br>humerus cancellous | 10<br>10 | 4.66<br>2.6 | 0.395<br>0.470 | |
| J. | 51 yr. | skull | 16 | 6.35 | 0.188 | |
| Sb. | 22 yr. | femur<br>tibia | 16<br>16 | 5.94<br>6.18 | 0.304<br>0.219 | 0.168<br>0.244 |
| F. | 37 yr. | iliac crest | 20 | 3.17 | 0.398 | |
| P. | 67 yr. | lumbar spinous process | 22 | 4.3 | 0.382 | |
| | | | Brain | | | |
| L. | 46 yr. | frontal lobe | 18½ | 2.12 | 0.600 | |
| | | | Cerebrospinal Fluid | | | |
| Sc. | 32 yr. | ventricle | 11 | —* | 0.967 | |
| B. | 8 mo. | lumbar | 20 | 3.305 | 0.953 | |
| G. | 54 yr. | ventricle | 20 | 3.320 | 1.005 | |

* Aliquot for sodium determination lost; specific activity calculated assuming ratio of spinal fluid/serum sodium to be 1.05.

suggested by a comparison of spinal fluid equilibration in subjects with normal and with elevated spinal fluid pressure; mixing was definitely delayed in the latter. In three normal subjects CFS/serum specific activity ratios ranged from 0.953 to 1.005 in the 11–20 hour period following radiosodium injection (see Table II), whereas in two subjects with increased intra-cranial pressure the ratio was 0.431 and 0.667 at the 12–16½ hour period.

Since studies in animals have revealed that radiosodium comes into equilibrium rather slowly with bone and brain sodium, and since the human skeleton is said to contain approximately 30% of the total sodium in the body (2), it was decided to study these two tissues. If equilibration is complete in bone and brain, it is presumably complete in other tissues. We are able to obtain only one specimen of normal brain tissue (at time of prefrontal lobotomy), but several specimens of bone were obtained at varying periods of time following radiosodium injection. Data with regard to bone, brain, and spinal fluid specific activity are presented in Table II. If chloride "space" may be taken as a measure of extracellular fluid in bone, our findings indicate that radiosodium mixes quite rapidly (within two hours) with the extracellular sodium of bone and confirm the results published by Kaltreider and his co-workers (8); however, our data indicate that it does not exchange readily with the remainder of the bone sodium. Definite conclusions would seem unwarranted in the face of so much scatter in the data, except to say that probably 40% of bone sodium exchanges with $Na^{24}$ over the period studied. Although the single value for brain specific activity is difficult to interpret, there seems to be no doubt that equilibration of $Na^{24}$ is complete in cerebrospinal fluid by 18–24 hours.

We present the data on bone with some hesitation because of the technical difficulties involved in the determination of bone sodium and the fact that there are so few published values with which to compare results. Everything possible was done to avoid contamination of the specimens at the operating table, yet the fondness of the surgeons for saline may have thwarted our efforts in some instances. Several of the samples came from elderly people and perhaps radiosodium mixing may occur more readily in the younger individuals who comprise the real subject material of this paper. Taken at face value, radiosodium equilibration in bone, except for sodium present in the chloride "space," must be extremely slow, and probably little could be gained, as far as total body sodium measurement is concerned, in prolonging the equilibration period beyond 18–24 hours. The short half-life of $Na^{24}$ would not permit the use of equilibration periods much longer than this without using larger doses and consequently delivering more radiation to the subject. Perhaps a large portion of bone sodium is truly "fixed," as the experiments of Harrison (18) would indicate, and not readily available for use in body fluid economy. Unfortunately, the literature contains no information on specific activity of bone sodium for comparison with ours. Since 30% of the total body sodium is said to be in bone (2), these data would suggest that about 82% of the total body sodium is measured by our method.[7]

The isotopic dilution method, therefore, measures that portion of the total body sodium which is most intimately concerned with the body fluid framework. "Exchangeable sodium," then, represents "mobile" or metabolically active sodium as contrasted to the structural sodium of the skeleton. Further study is needed to determine the exact magnitude of the non-exchangeable portion of the body sodium and the precise relationship of TES to total body sodium in man.

## SUMMARY

*1)* Measurements of total "exchangeable sodium" in 32 healthy young adults by the isotopic dilution method (utilizing $radiosodium^{24}$) revealed an average value of 41.9 meq. and 39.5 meq. per Kg. of body weight for males and females, *respectively*, with a range of from 32 to 54 meq. per Kg. in males and from 36 to 42 meq. in females.

*2)* An 18–24 hour equilibration period following $Na^{24}$ administration was selected as optimum on the basis of (*a*) the shape of the equilibration curve and (*b*) tissue studies.

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[7] Brain contains only 3% of the body sodium (2).

3) Determination of sodium specific activity in bone suggests that as much as 60% of the skeletal sodium may be metabolically inert, for it does not exchange with Na$^{24}$ during the equilibration period.

4) It was decided to designate the measured quantity as total "exchangeable sodium." The isotopic dilution method, therefore, measures that portion of the body sodium most intimately concerned with fluid balance. By indirect means, it was estimated that TES represents at least 82% of the actual amount of sodium in the adult body.

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# ESTIMATION OF TOTAL BODY SODIUM BY ISOTOPIC DILUTION. II. STUDIES ON INFANTS AND CHILDREN: AN EXAMPLE OF A CONSTANT DIFFERENTIAL GROWTH RATIO[1, 2]

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(Submitted for publication December 13, 1950; accepted, March 19, 1951)

In contrast to the lack of such information on adults, the literature does contain reports of the direct chemical analysis of a number of fetuses and newborn infants for sodium. Data are available, therefore, with which to compare directly results obtained by the isotopic dilution method. Our study of total body sodium in man, for which data on adults are presented elsewhere (2), was consequently broadened to include infants and children. Since it has been known for some time that the sodium content of the infant, on a per kilogram basis, is higher than that of the adult (3), we set out to investigate the possibility that there was some regularity to the process of chemical growth. That a chemical growth pattern, capable of being expressed in simple mathematical terms, does indeed exist, at least with regard to sodium accumulation, will be demonstrated by the data to follow.

Aside from carcass analysis, the isotopic dilution method is the only direct approach available for estimation of total body sodium and for the study of chemical growth. Cumulative balances fail to provide a satisfactory answer, presumably through inability to account accurately for cutaneous mineral losses. For example, balance studies on normal infants and children have yielded values for sodium retention varying from 93 to 915 meq. per Kg. of body weight gain (4, 5).[4]

This paper reports our studies of the estimation of total body sodium by the isotopic dilution method in 21 infants and children. The various assumptions which had to be made in applying this method to the estimation of total body sodium in man have already been discussed (2).

## METHODS

The methods used are essentially those employed in the studies of adults (2). Radiosodium$^{24}$, manufactured in the Washington University cyclotron, and prepared in our laboratory as sterile isotonic saline, was given intravenously in doses of 1 to 1½ microcuries per Kg. of body weight.[5]

Procedures used in assaying the serum and urine samples for radiosodium and inactive sodium have already been presented (2).

The subjects employed were infants and children residing in the wards of the Saint Louis Children's Hospital, whose general nutritional and physical status was judged to be normal and who were free from cardiac, renal, or allergic disease. No patients who were convalescing from surgical operations or who were receiving parenteral fluid therapy were included; none had loose stools. They consumed the normal hospital diet (evaporated milk-Karo formulas in the case of infants) during the experiments. Reasons for hospitalization are listed in Table I to follow.

Calculations were made as follows: serum obtained 18–24 hours (17 hours in three infants) following the intravenous administration of a known amount of radiosodium was analyzed for sodium and radioactive sodium, and urine was collected over the same interval of time. Assuming complete equilibration:

$$\text{total exchangeable sodium (TES)} = \frac{\text{Na}^{24}\text{ injected} - \text{Na}^{24}\text{ excreted}}{\text{serum Na}^{24}/\text{serum sodium}}.$$

This period of equilibration was chosen for reasons presented elsewhere (2). The amount of sodium administered with the radiosodium is negligible. No attempt was made to take into account the Donnan phenomenon, slight

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[1] This work was carried out under grants from the Children's Research Foundation and the U. S. Atomic Energy Commission.

[2] A preliminary report of these studies has been published elsewhere (1).

[3] Present address: Southwestern Medical School, Dallas, Texas.

[4] Calculated from the author's data.

[5] The total radiation dose received by the experimental subject from this amount of Na$^{24}$ is of the order of 0.11 to 0.17 roentgen equivalent physical (6). This calculation was made for subjects of adult size; since gamma radiation accounts for approximately 75% of the total dose, it is possible that recalculation of the amount of radiation received by the young infant would yield a lower value.

differences in diffusibility between $Na^{24}$ and $Na^{23}$ ions, or the possibility that small amounts of radiosodium might be excreted by way of the skin or gastrointestinal tract.

## RESULTS

The results obtained during a study of 21 healthy infants and children are presented in Table I, in which the subjects are listed in order, according to weight. Total exchangeable sodium has been calculated on the basis of weight, height, and surface area. None of these result in constant unit values for TES, for the values change throughout the growing period in each instance. The difference between infants and older children is most marked on the basis of height, the values indicating a four-fold increase of total body sodium per centimeter of height, whereas sodium content per square meter of surface area increased by only 25% (approximately) over the span of growth represented by our subjects. The trend in the data is opposite in direction when calculations are made on the basis of weight. In the very young infant, values are highest, averaging 76 meq. sodium per Kg. of body weight, and there is a progressive decline with increasing weight until adult values (41.9 meq. per Kg. for males, 39.5 meq. per Kg. for females [2]) are attained. Since sodium content is constantly changing with growth regardless of whether weight, height, or surface area is employed as a reference, body mass was selected as the logical parameter of body size with which to attempt to correlate sodium content. It will be shown, in the discussion to follow, that there is a recognizable mathematical relation, not necessarily dependent on age, between the amount of sodium in the body, and body mass, as measured by weight.

## DISCUSSION

In the study of adult subjects (2) it was realized that mixing of injected $Na^{24}$ with body sodium may not be quite complete at the end of 18–24 hours, although curves of the apparent volume of $Na^{24}$ distribution and of serum specific activity revealed a leveling off at that time. Moreover, it was concluded that prolongation of the equilibration period would be of little benefit and, in fact, might intensify errors due to extrarenal losses of $Na^{24}$. Studies of bone specific activity revealed that complete $Na^{24}$ mixing in bone

TABLE I

*Total exchangeable sodium in infants and children*

| Subject | Age | Sex | Weight | Height | Surface area | Equilibration time | TES | Meq. per Kg. | Meq. per cm. | Meq. per m² | Reason for hospitalization |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | *Gm.* | *cm.* | *m²* | *hours* | *meq.* | | | | |
| G. S. | 2 wks. | M | 1795 | 46 | 0.149 | 19 | 148 | 82.5 | 3.22 | 994 | Premature |
| R. C. | 6 wks. | M | 2155 | 47 | 0.162 | 20 | 187 | 86.8 | 3.98 | 1155 | Premature |
| R. G. | 2½ wks. | M | 2375 | 48 | 0.172 | 20 | 187 | 78.7 | 3.90 | 1088 | Premature |
| A. J. | 3 wks. | M | 2590 | 45 | 0.170 | 17½ | 184 | 71.2 | 4.09 | 1082 | Premature |
| G. E. | 4 wks. | M | 3530 | 53 | 0.218 | 21 | 259 | 73.4 | 4.89 | 1188 | Methemoglobinemia; recovered |
| L. L. | 2 mo. | F | 3665 | 55 | 0.230 | 17 | 242 | 66.0 | 4.40 | 1053 | Ruminator |
| E. H. | 2½ mo. | M | 4870 | 59 | 0.272 | 17 | 336 | 69.0 | 5.70 | 1237 | Roseola infantum; recovered |
| G. J. | 14 mo. | M | 6960 | 68 | 0.348 | 23½ | 364 | 52.3 | 5.20 | 1018 | Premature infant ? lye burns of esophagus |
| R. C. | 4¼ mo. | M | 7200 | 67 | 0.350 | 17½ | 406 | 56.4 | 6.05 | 1160 | ? skull fracture |
| L. O. | 12 mo. | M | 11,480 | 84 | 0.503 | 18 | 564 | 49.1 | 6.60 | 1100 | Mental defective |
| S. M. | 22 mo. | M | 11,630 | 81 | 0.490 | 23 | 504 | 43.4 | 6.21 | 1030 | ? hydrocephalus |
| J. D. | 13 mo. | M | 12,750 | 82 | 0.515 | 18½ | 691 | 54.4 | 8.44 | 1340 | Osteomyelitis spine |
| R. H. | 3⅓ yrs. | M | 14,700 | 99 | 0.620 | 18 | 847 | 57.6 | 8.56 | 1365 | Tbc suspect |
| E. R. | 3 yrs. | M | 15,400 | 103 | 0.660 | 19½ | 863 | 56.1 | 8.37 | 1308 | Hernia, pre-op. |
| B. T. | 3½ yrs. | M | 18,400 | 101 | 0.700 | 17 | 938 | 51.0 | 9.30 | 1340 | Hematoma forehead |
| J. H. | 6 yrs. | M | 21,100 | 123 | 0.860 | 18 | 1107 | 52.4 | 9.00 | 1290 | Tbc suspect |
| C. M. | 10 yrs. | F | 23,800 | 137 | 0.970 | 19½ | 1163 | 48.9 | 8.50 | 1200 | ? chorea |
| J. K. | 11 yrs. | M | 31,100 | 139 | 1.10 | 18 | 1790 | 57.6 | 8.44 | 1630 | Idiopathic epilepsy |
| G. B. | 14 yrs. | M | 37,600 | 146 | 1.25 | 23 | 1478 | 39.3 | 10.2 | 1190 | ? coarctation aorta |
| J. P. | 13 yrs. | M | 39,000 | 158 | 1.33 | 18 | 1992 | 51.0 | 12.6 | 1500 | Behaviorism |
| M. K. | 11 yrs. | F | 52,800 | 165 | 1.56 | 23 | 1862 | 35.2 | 11.3 | 1193 | Epilepsy, idiopathic |
| L. M. | 14 yrs. | M | 56,800 | 165 | 1.61 | 20 | 2440 | 43.0 | 14.8 | 1515 | Small *osteosarcoma*, tibia |

TABLE II

*Comparison of total body sodium measured by direct chemical analysis* and by isotopic dilution in young infants, meq. per Kg.*

| Chemical analysis* | | | | | Isotopic dilution | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Number of subjects | Weight range, *Gm.* | Meq. per Kg. | | | Number of subjects | Weight range, *Gm.* | Meq. per Kg. | | |
| | | Max. | Min. | Average | | | Max. | Min. | Average |
| 11 | 2476–3360 | 93.0 | 58.5 | 75.9 | 6 | 1795–3665 | 86.8 | 66.0 | 76.4 |

* Calculated from the literature (7–11).

would probably necessitate prolonging the equilibration period considerably; unfortunately only one of the samples analyzed for specific activity was from a child. About 40% of the bone sodium would appear to be exchanged at the end of 18–24 hours in the adult; however, the difficulties encountered in determining the specific activity of bone sodium make us hesitate to place too much reliance on this value without further study.

TABLE III

*Total body sodium in the human fetus*

| Reference | Weight | Fetal age, lunar months | Total Na | Na per Kg. |
|---|---|---|---|---|
| | *Gm.* | | *meq.* | *meq.* |
| (7) | 18.2 | 3.0 | 0.9 | 50.0 |
| (7) | 23.9 | 3.2 | 0.8 | 34.5 |
| (7) | 59.4 | 3.7 | 4.5 | 76.4 |
| (7) | 114.5 | 4.3 | 11.6 | 101.1 |
| (7) | 259.0 | 5.1 | 29.9 | 103.7 |
| (7) | 335.0 | 5.4 | 31.3 | 94.4 |
| (7) | 490.0 | 5.7 | 50.5 | 103.3 |
| (8) | 522.0 | 4.5–5.0 | 55.4 | 106.0 |
| (8) | 570.0 | 5.0–5.5 | 67.5 | 118.5 |
| (7) | 570.0 | 6.4 | 53.5 | 94.4 |
| (7) | 590.0 | 6.2 | 55.9 | 95.3 |
| (8) | 800.0 | 5.5–6.0 | 74.4 | 93.0 |
| (7) | 960.0 | 7.6 | 98.4 | 102.5 |
| (12) | 970.0 | 6.7 | 105.0 | 108.5 |
| (7) | 1010.0 | 7.2 | 83.7 | 83.0 |
| (8) | 1165.0 | 6.7 | 105.5 | 90.5 |
| (7) | 1205.0 | 7.7 | 104.8 | 87.6 |
| (8) | 1285.0 | 7.2 | 116.1 | 90.5 |
| (12) | 1420.0 | 6.7–7.8 | 134.0 | 94.5 |
| (7) | 1545.0 | 8.2 | 136.0 | 88.5 |
| (7) | 1555.0 | 8.2 | 127.2 | 82.0 |
| (7) | 1615.0 | 8.4 | 143.3 | 89.2 |
| (11) | 2476.0 | Term | 200.0 | 80.9 |
| (11) | 2616.0 | Term | 176.0 | 67.4 |
| (9) | 2680.0 | Term | 225.0 | 84.0 |
| (11) | 2683.0 | Term | 179.5 | 66.9 |
| (8) | 2720.0 | Term | 253.0 | 93.0 |
| (11) | 2755.0 | Term | 196.0 | 71.3 |
| (7) | 2915.0 | Term | 240.4 | 76.2 |
| (11) | 3080.0 | Term | 284.0 | 92.4 |
| (10) | 3211.0 | Term | 217.5 | 67.7 |
| (8) | 3300.0 | Term | 193.0 | 58.5 |
| (11) | 3360.0 | Term | 259.0 | 77.1 |

The literature contains reports of sodium analysis of the complete carcass (in many the meconium was removed) of 11 term fetuses. Comparison with these data provides the only way in which our own measurements can be directly evaluated. Values obtained by the two methods are presented in condensed form in Table II, in which the analyses for term fetuses from the literature are set against our analyses (by isotopic dilution) for young infants. Agreement between these two groups of roughly comparable weight is surprisingly good, and suggests that total sodium can be measured, at least in the young infant, by the isotopic dilution method. Most of the sodium of the infant, according to this comparison, would be exchangeable with radiosodium. This is in contrast to the adult, in whom as much as 18% of the body sodium may escape detection by the isotopic dilution method (2). In the absence of data on bone specific activity in infants and children we shall continue to designate the quantity of sodium measured by our method as total "exchangeable sodium."

*Change in total body sodium with age.* In Table III are listed data from the literature in regard to the direct chemical analyses of 33 fetuses and newborns for sodium. The progression of values for total body sodium is in the same direction as we found it to be in infants and children; except for the three youngest fetuses, total body sodium declines steadily, on a per kilogram basis, with advance in fetal age.

All of the data available on total body sodium have been combined in Figure 1 in which the sodium content, in meq. per Kg., is plotted against age for an age range from the third month of fetal life to the 34th year of post-natal life. Sodium content is quite low during the early months of fetal life (34–50 meq. per Kg.), but rises rapidly to a maximum (94–118 meq. per Kg.) in the five month fetus, after which it falls, at first quite rapidly, later more slowly, to adult values at about the time of puberty. Interestingly enough, adult values are quite similar to those noted for the two youngest fetuses. We had thought at one point in our investigations that experimental error might be responsible for the rather wide range of values noted in our supposedly healthy subjects; the spread in values reported for direct carcass analysis, a method which should be less subject to experimental error than is the isotopic dilution one, was reassuring.

*Relation of total body sodium to body weight.* Organization of the available data on total body sodium (Tables I and III, and Table I of reference 2) is achieved by plotting sodium content against weight on a double logarithmic grid. Values obtained by direct chemical analysis (from the literature) are illustrated in Figure 2. Two regression equations are necessary to describe sodium growth from the third month of fetal life to birth; calculated by the method of least squares, these are, for the 18–115 Gm. fetus:

$$\log \text{Na content, meq.} = 1.51 \log \text{weight, Gm.} - 2.044.^{6} \qquad (1)$$

or in exponential form

$$\text{Na, meq.} = 0.00904\ \text{Gm.}^{1.51}$$

and for the 259–3360 Gm. fetus:

$$\log \text{Na} = 0.832 \log \text{weight} - 0.537 \qquad (2)$$

or

$$\text{Na} = 0.290\ \text{Gm.}^{0.832}$$

Whether the change in slope from equation *1*, indicating that sodium content of the fetus is increasing rapidly on a per kilogram basis, to that of equation *2*, in which the reverse obtains, is abrupt or gradual cannot be ascertained from the data at hand.

Data for post-natal life, obtained by the isotopic dilution method, are plotted in a similar manner in Figure 3. The regression equation for in-

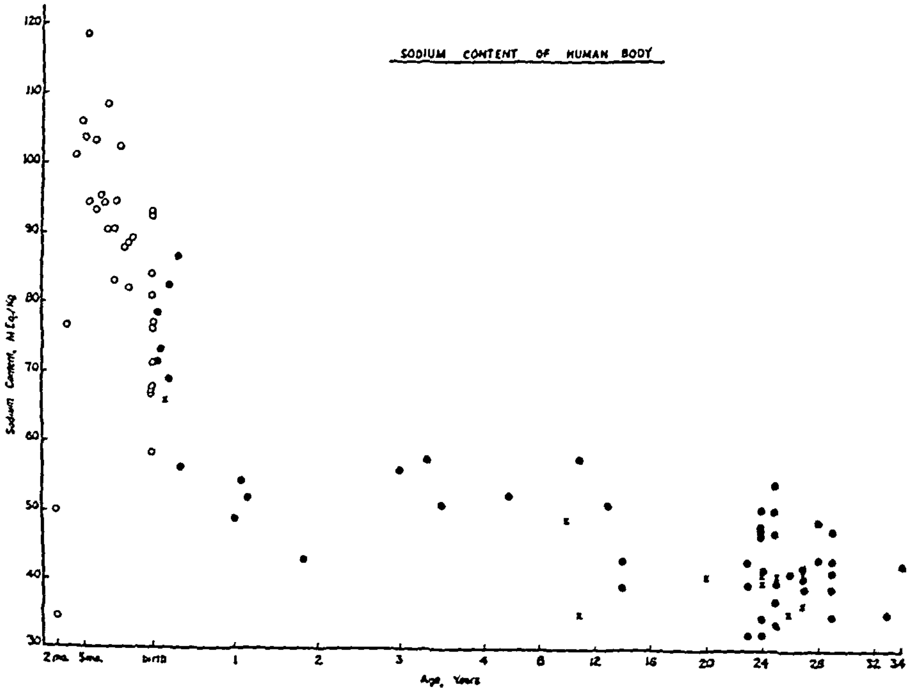

FIG. 1. PLOT OF SODIUM CONTENT, MEQ. PER KG., AGAINST AGE IN YEARS

○ = fetus, total body sodium by direct chemical analysis (Table III). Infants, children, and adults, TES by isotopic dilution (Table I and Table I of reference 2): ● = males, × = females.

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[6] Logarithms to the base 10.

fants,[7] children and adult males is strikingly similar to that calculated for the older fetuses. The following equation fits these data quite well over a weight range of 1795–100,000 Gm.:

$$\log \text{Na} = 0.829 \log \text{weight} - 0.552 \qquad (3)$$

or

$$\text{Na} = 0.281 \ \text{Gm.}^{0.829}$$

For females between 23,800 and 81,600 Gm. in weight, the equation is

$$\log \text{Na} = 0.754 \log \text{weight} - 0.234 \qquad (4)$$

or

$$\text{Na} = 0.584 \ \text{Gm.}^{0.754}$$

The various statistical constants for these equations are listed in Table IV. In view of the

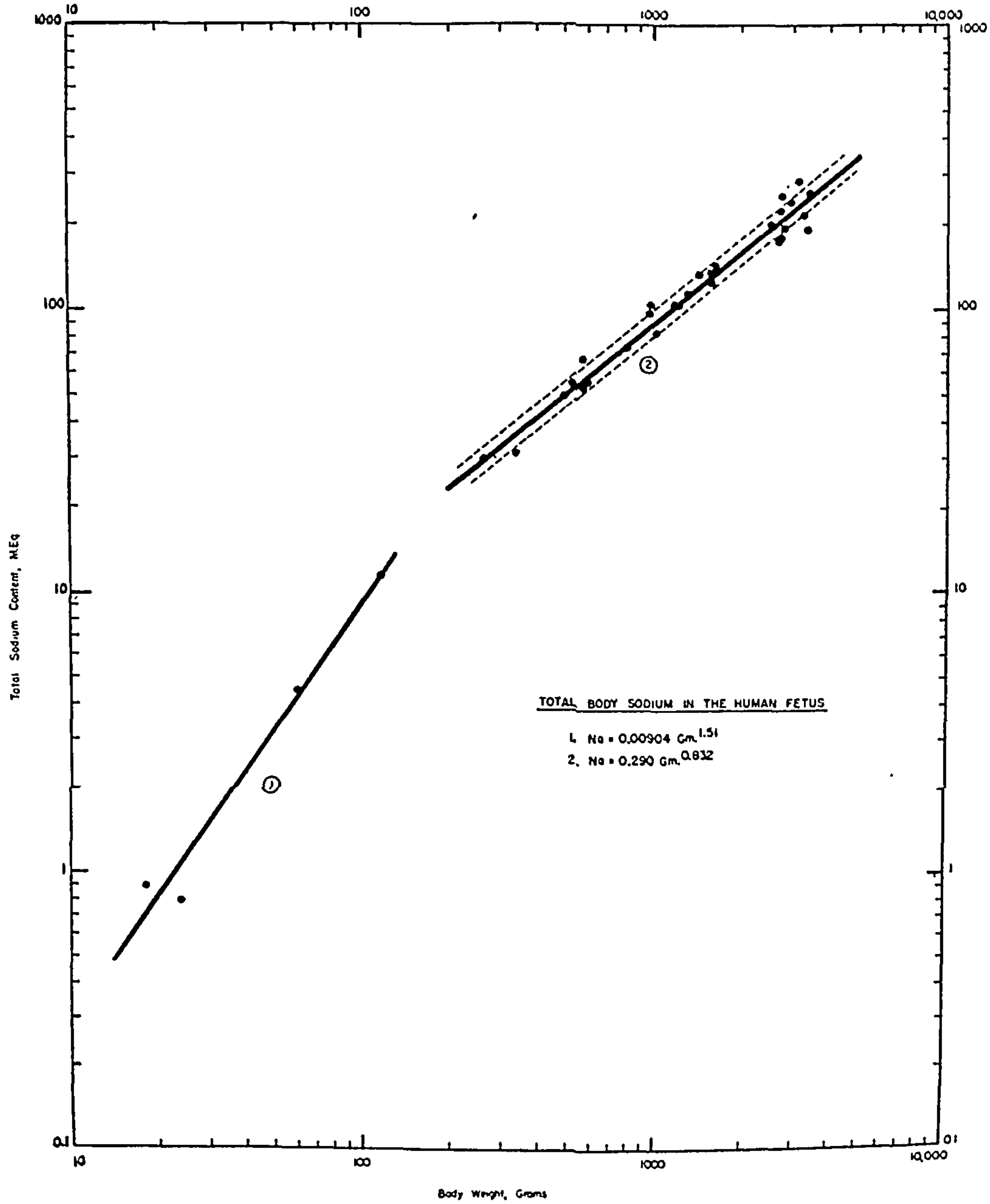

FIG. 2. LOG-LOG PLOT OF TOTAL BODY SODIUM AGAINST WEIGHT FOR THE HUMAN FETUS

Data from Table III. Regression lines drawn according to equations *1* and *2*. The dotted lines represent one standard deviation from regression.

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[7] With one exception, all of the infants in our series were males. The single female infant was so far removed from the main group of female subjects (Table I) that its inclusion in the regression calculations for females would weight the resulting equation rather heavily in its favor; it was therefore grouped with the males.

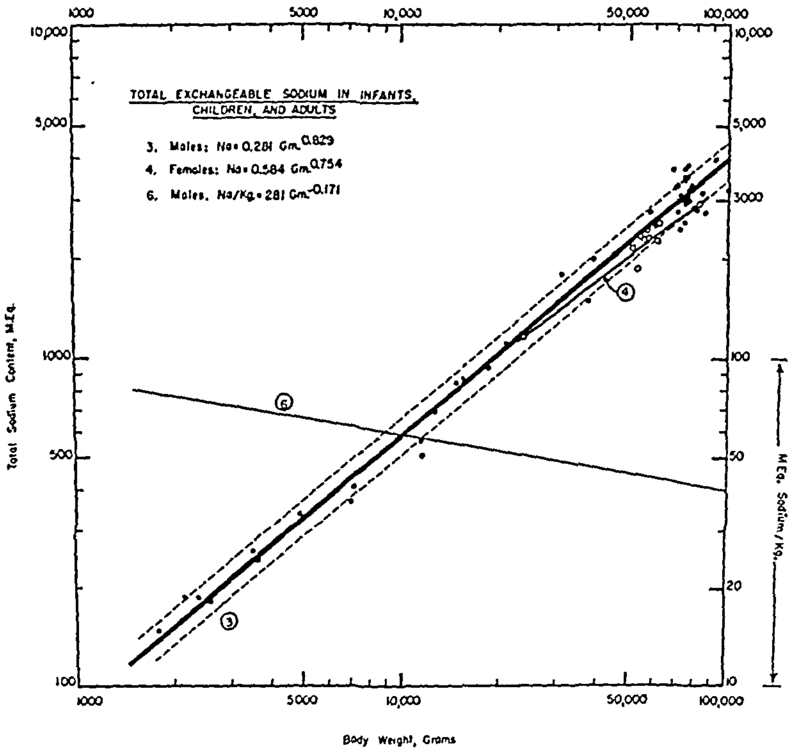

FIG. 3. LOG-LOG PLOT OF TES AGAINST WEIGHT FOR INFANTS, CHILDREN, AND ADULTS

Data from Table I and Table I of reference 2. Regression lines drawn according to equations *3*, *4*, and *6*. Dotted lines represent one standard deviation from regression (equation *3*). Notice that the lower half of the right-hand vertical scale has been changed to allow for inclusion of the regression line for equation *6*. ● = males, ○ = females.

small number of observations, they are omitted for equation *1*.

That there is an orderliness to chemical growth is immediately apparent; furthermore, the growth pattern can be expressed by a simple exponential equation, and it does not seem to be necessary to resort to more complicated expressions such as the quintic used by Mitchell and associates (13) to describe calcium growth in the human. The regression lines described by equations *2* and *3* are virtually parallel, and extrapolation of either one would cause it to lie within one standard deviation of the other over the range of body weights under study. Log-log plots of sodium content against height and surface area were also made, but the fit of the data (by inspection) was no better than that using weight. Logically, weight is to be preferred over height or surface area as a basis of reference, since weight and sodium are both measured in mass units.

TABLE IV

| Subjects | Number | Weight range, *Gm* | k | Standard error of k | Standard deviation of regression | Correlation coefficient |
|---|---|---|---|---|---|---|
| Fetuses (equation *2*) | *29* | *259–3360* | *0.832* | *0.027* | *0.047* | *0.988* |
| Infants, children and adult males (equation *3*) | 49 | 1795–100,000 | 0.829 | 0.015 | 0.057 | 0.992 |
| Females (equation *4*) | 9 | 23,800–81,600 | 0.754 | 0.007 | 0.028 | 0.972 |

Despite the smooth trend of the data, attested by the excellent correlation coefficients (Table IV), they are not sufficient to allow formal statistical comparison between the various regression equations. The data are not distributed homogeneously within each group, and the range covered by the female data (equation *4*) is too small. The largest group of subjects are described by equations *2* and *3*, whose slopes are almost parallel; yet the two regression lines overlap during only a small part of the total growth period (1795–3360 Gm.).

*Characterization of total body sodium by means of a constant differential growth ratio.* Extrapolation of the fetal data (equation *2*) to cover the range represented by adults (equation *3*) is certainly unwarranted on a statistical basis, yet the similarity between the two regression coefficients is of considerable interest.

The data suggest very strongly that a constant differential growth ratio characterizes sodium "growth" in man from mid-fetal life to maturity. First used a half-century ago to relate brain weight to total body weight (*vide* Brody [14] pp. 605–609), the concept of relative growth rates was expanded by Huxley (15) and shown to apply to individual organ weights and lengths in a number of species. Brody (14) demonstrated that the concept could be extended beyond the realm of morphology to include a number of physiological functions, while Needham (16) called attention to the fact that chemical growth could also be characterized in this way. The general equation is an exponential one, $y = bW^k$. Using symbols appropriate to this report we have

$$Na = bW^k \qquad (5)$$

where Na is total body sodium or total "exchangeable sodium" in meq. depending on which of the foregoing equations are used, and W is body weight in Gm. The general equation states, in essence, that the specific growth rate of an organ or in this instance a body constituent, $\frac{1}{Na} \cdot \frac{dNa}{dt}$, is directly proportional to the specific growth rate of the body as a whole, $\frac{1}{W} \cdot \frac{dW}{dt}$.[8] The close fit of the data noted in Figures 2 and 3 indicates that equation *5* can be used to describe the process of chemical growth in man quite nicely, and consequently that the rate of sodium accumulation per unit total body sodium is directly proportional to the rate of increase in body weight per unit weight during growth. The exponent *k*, which is independent of the units of measurement employed, is the ratio of differential growth. The data presented in this report indicate that *k* is constant in the human organism between weights of 250–100,000 Gm.

Equation *5* states that body sodium content does not vary directly with simple body weight but with body weight raised to the *k*th power. Brody proposed that $W^k$ be designated "physiological weight" or "metabolically effective weight" in contradistinction to $W^{1.0}$ which is body mass or gravitational weight. Total body sodium increases during growth as a constant fractional power of body weight: in terms of *per cent change,* body sodium content increases by only 83% for each 100% change in weight. A consideration of equations *2* and *3* indicates that this "fundamental chemical plan of animal growth" (16) is in operation over most of the span of human growth.

Data on total body sodium content have been reported for the chick embryo (17) and for the young rat (18). The ratio of differential growth, *k*, remains constant in the chick embryo at the value of 0.94 between four and 21 days of incubation, and for the rat at 0.812 over the period studied, from birth to 28 days.[9] The latter ratio is very close to that for the human; it is unlikely, therefore, that the demonstrated mathematical relationship between sodium content and body weight in man is a fortuitous one. Although objections have been raised to the use of such a simple mathematical expression for describing growth (19), it has proved very useful in interpreting a variety of growth phenomena in different species and in providing a basis for interspecies comparison (14).

A comparison of the regression equations for fetuses (equation *2*) and for infants, children, and adults males (equation *3*) suggests that there may be systematic error in the isotopic dilution method for determination of total body sodium in that values appear to be slightly lower than would be predicted from extrapolation of data obtained by direct chemical analysis. If it is assumed that a comparison between these two equations is warranted, mean values for total "exchangeable sodium" represent 93% of total body sodium in the infant of 3000 Gm. and 91% of the total sodium in the 100 Kg. adult male. The close similarity of the regression coefficients for equations *2* and *3* in some degree justifies the extrapolation of the fetal data; however, the question of the exact magnitude of the relationship of TES to the actual amount of sodium in the body cannot be settled until complete carcass analyses have been made in the older child and adult.

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[8] *Specific growth rate* is defined as the rate of change of log body size with respect to time, $\frac{d(\log W)}{dt}$, in contrast to *simple growth rate,* $\frac{dW}{dt}$.

[9] The value of *k* was determined by inspection in the case of the chick data and by calculation for the rats.

*Predicted values for total "exchangeable sodium."* The equations presented above allow one to predict the mean value for sodium content for any group of individuals of given weight. Total body sodium can be predicted for the human fetus 18 Gm. or more in weight by the use of equations *1* and *2*, and total "exchangeable sodium" in post-natal life for subjects whose body weights are between 1800 and 100,000 Gm. in weight by substitution in equation *3* and *4*.

The mean content of sodium, in terms of meq. per Kg. body weight, can be calculated directly if equations *1–4* are written in a slightly different way. Using equation *3* as an example, and converting to kilograms, we have

$$\text{meq. Na (TES)/Kg.} = 281 \text{ weight, Gm.}^{-0.171} \qquad (6)$$

A plot of equation *6* is included in Figure 3, from which can be read directly the mean total exchangeable sodium, meq. per Kg., for any given body weight from 1795 to 100,000 Gm. There is, therefore, a steady and predictable decline in sodium content per unit weight during growth. Our data are not sufficient to permit statements as to the magnitude of the deviation from this curve which could be considered abnormal for any given subject.

*The sodium increment of normal growth.* Differentiation of the relative growth equation, $\text{Na} = \text{bW}^{\text{k}}$, with respect to weight, yields the following:

$$\text{d (Na)/dW} = \text{k Na/W} \qquad (7)$$

The rate of change of sodium content with respect to weight is therefore a function of the amount of sodium in the body, in terms of meq. per unit weight, and would be expected to decline as growth proceeds since $k$ is less than unity. From this equation the *instantaneous* rate of change in body sodium per unit body weight *gain* can be calculated for any given body weight. As a result, one can anticipate the sodium increment due to normal growth from a knowledge of initial weight and weight gain provided the observational period is not too long. For example, an infant of 4000 Gm. (predicted sodium content 0.068 meq. per Gm.) would be expected to retain $0.068 \times 0.829 = 0.056$ meq. of sodium for each gram of weight gain as a result of normal accumulation of new body tissue. A gain of 20 Gm. in body weight on this particular day of observation would therefore lead to a positive sodium balance of 1.1 meq. Perhaps calculations done in this way could be of some use in the interpretation of balance studies.

*Correlation of total sodium content with other body components.* A number of organ weights in man have been related to total body weight by the use of the relative growth equation (Brody [14], p. 584). One can therefore calculate the relative growth rate of a given body component in terms of a second component in the way suggested by Adolph (*20*).

The importance of the kidney in body sodium economy is brought out most clearly by this sort of approach. During post-natal life of all the organs listed for man, only kidney weight changes as the 0.83 power of body weight, the equation being $y = 0.00892 \text{ W}^{0.83}$, where $y$ and $W$ stand for kidney weight and total body weight, respectively, in grams. Since total body sodium (or TES) also changes as the 0.83 power of body weight during growth, there is an arithmetic relationship between kidney weight and the amount of sodium in the body. Division of equation *3* by the equation for the kidney weight yields the following:

$$\frac{\text{TES, meq.}}{\text{kidney weight, Gm.}} = \frac{0.281}{0.00892} = 31.5$$

which suggests that each gram of kidney tissue supports in the body economy about 31.5 meq. of "exchangeable" sodium. Renal function *per se* is not considered here; rather it is the relation of absolute kidney weight to the amount of sodium present in the body. The ratio is applicable (at least in males) over a large range of body weight. While this type of mathematical approach does not constitute proof of the suggested relationship between sodium content and kidney size, it does serve to emphasize further the intimate role of the kidney in sodium metabolism. Data for the mass of two other body components concerned in sodium metabolism, namely, skeleton and muscle, unfortunately are not available for comparison with total sodium content in this way.

## SUMMARY

*1*) Measurements of total "exchangeable sodium" (TES) were made in a series of 21 infants and children by the isotopic dilution method, using radiosodium$^{24}$. The subjects ranged in weight from 1795 to 56,800 Gm.

*2*) A comparison of the data for young infants with carcass analyses reported in the literature indicates that the two methods for determining total body sodium give comparable results.

*3*) Values for TES are highest in the young infant, averaging 70 meq. per Kg. in the 3300 Gm. newborn and gradually decline in a curvilinear fashion with age until adult values are reached (approximately 42 meq. per Kg.).

*4*) When all of the available data on total body sodium (TES data from this report and that of the preceding report [2] together with carcass analyses from the literature) are brought together, a straight line relationship results when sodium content is plotted against body weight on a double logarithmic grid.

*5*) Total body sodium thus conforms to the relative growth equation, $Na = bW^k$, from midfetal life to maturity. Regression equations have been calculated which allow mean values of total body sodium or TES to be predicted for any given weight.

*6*) Treatment of the data in this way suggests several tentative conclusions:

*a*) Total sodium changes with body weight in a predictable systematic fashion, namely, as a fractional power of body weight, and behaves as a constant differential growth ratio.

*b*) Predictions of the sodium retention occurring as a result of normal growth can be made for any given weight.

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# THE IMMUNOPHYSIOLOGY OF TRICHINOSIS. ALTERATIONS IN THE BLOOD VOLUME AND THE THIOCYANATE SPACE IN RELATION TO THE DEVELOPMENT OF HUMORAL ANTIBODIES IN THE RABBIT[1]

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(Submitted for publication January 22, 1951; accepted, March 19, 1951)

The development of edema during the course of trichinosis has been observed in patients for many years. The accumulation of fluid, especially around the eyes, has been recognized as one of the most frequent clinical manifestations of the disease. The mechanism by which edema develops during the course of this and other acute infectious diseases has been obscure.

During the course of studies on patients with Rocky Mountain spotted fever, it was observed that alterations in the distribution of body fluids with the development of marked clinical edema reached a maximum just before clinical recovery begins (1). Observations on one patient who had been given hyperimmune rabbit antiserum for Rocky Mountain spotted fever showed that the alterations in the blood volume and thiocyanate space, which occurred during classic serum sickness following recovery from the initial disease, resembled strikingly those seen in the same patient during the acute stage of the rickettsial infection. These observations suggested the possibility that an immune reaction may be directly or indirectly responsible for the changes in the permeability of membranes which permit alteration in the fluid distribution in rickettsial spotted fever.

In subsequent studies on experimental serum sickness in rabbits, the alterations in the distribution of body fluids were found to resemble those encountered in patients with spotted fever (2). The time relationship between the maximal alterations and the appearance of circulating antibodies (precipitins) was interpreted as indicating that the increase in permeability of membranes was the result of an antigen-antibody reaction.

The present experiments were undertaken to determine the magnitude of changes in the blood volume and thiocyanate space which occur during an *interstitial* infection such as trichinosis (as contrasted to the *intracellular* infection of spotted fever and the antigen-antibody reaction induced *without* infection of serum sickness) and to correlate the physiologic alterations with the development of humoral immunity.

## MATERIAL

Domestic rabbits of mixed breeds, weighing 2 to 4 kilograms each, were placed in individual cages and fed a stock diet (Kasco Complete Rabbit Ration, Kasco Mills, Inc., Toledo, Ohio), supplemented once weekly by fresh green vegetables. Water was given without restriction.

The strain of *Trichinella spiralis* used was obtained from the National Institute of Health and was carried in white rats. The rats were killed six weeks after infection, and the skeletal muscle was pooled and chopped fine with scissors. A sample of the pooled meat was examined in a muscle press and found to contain approximately 120 encysted muscle trichinae per Gm. It was fed to the rabbits within 24 hours.

## METHODS

The details of the chemical techniques and experimental procedure for fluid volume determinations in rabbits have been previously published (2, 3).

*Immunologic technique:* The presence of circulating humoral antibody was detected by the ring precipitin test performed in 2 × 20 mm. tubes. Undiluted rabbit serum (antibody) was overlayed with a 1:200 dilution in saline of powdered muscle trichinae (antigen) prepared by Bozicevich's method (4).

*Plan of the experiment:* Determinations of the blood volume, thiocyanate space and precipitin tests were done simultaneously every second or third day. A minimum of eight determinations was done on each animal. The two groups were treated in exactly the same fashion except for the feeding of trichinous rat meat. Uninfected meat was not fed to the rabbits in group 1, since previous experiments had failed to demonstrate that the feeding of uninfected rat meat can produce sensitization to foreign proteins (5). The six control animals (group 1) were followed at least 30 days.

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[1] This study was aided by a grant from the John and Mary R. Markle Foundation.

TABLE I

*Alterations in fluid space in rabbits ill with trichinosis (group 2)*

| Rabbit number | Baseline | | | | | | | | | | Dose | Clinical severity | Response* | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | I | | | | | II | | | | | | | I | | | | | | II | | | | | |
| | Weight | Thio-cyanate space | Plasma volume | Total blood volume | Hema-tocrit | Weight | Thio-cyanate space | Plasma volume | Total blood volume | Hema-tocrit | | | Day† | Weight | Thio-cyanate space | Plasma volume | Total blood volume | Hema-tocrit | Day | Weight | Thio-cyanate space | Plasma volume | Total blood volume | Hema-tocrit |
| | *kilo.* | *ml.* | *ml.* | *ml.* | *vols. %* | *kilo.* | *ml.* | *ml.* | *ml.* | *vols. %* | *Gm.* | | | *kilo.* | *ml.* | *ml.* | *ml.* | *vols. %* | | *kilo.* | *ml.* | *ml.* | *ml.* | *vols. %* |
| 7 | 1.99 | 443 | 130 | 213 | 39.0 | 2.21 | 480 | 124 | 194 | 36.0 | 5.0‡ | Moderate | 16 | 2.16 | 485 | 121 | 202 | 40.0 | 19 | 2.16 | 544 | 102 | 162 | 37.0 |
| 8 | 2.56 | 431 | 117 | 202 | 42.0 | 2.50 | 412 | 122 | 188 | 35.0 | 6.0‡ | Severe | 14 | 2.61 | 770 | 109 | 170 | 36.0 | 17 | 2.67 | 725 | 132 | 215 | 38.5 |
| 9 | 2.21 | 475 | 98 | 163 | 40.0 | 2.27 | 432 | 98 | 163 | 40.0 | 5.5‡ | Moderate | 16 | 2.38 | 598 | 123 | 188 | 35.0 | 19 | 2.50 | 472 | 174 | 268 | 35.0 |
| 10 | 3.24 | 512 | 127 | 223 | 43.0 | 3.24 | 635 | 131 | 223 | 41.0 | 6.0 | Mild | 16 | 3.24 | 672 | 107 | 176 | 39.0 | 20 | 3.35 | 668 | 120 | 202 | 40.5 |
| 11 | 2.67 | 650 | 123 | 212 | 42.0 | 2.67 | 653 | 154 | 230 | 33.0 | 3.0 | Moderate | 12 | 2.78 | 708 | 122 | 185 | 34.0 | 15 | 2.78 | 560 | 121 | 206 | 41.0 |
| 12 | 2.95 | 650 | 127 | 213 | 40.0 | 2.78 | 658 | 119 | 186 | 36.0 | 3.0 | Moderate | 21 | 3.07 | 670 | 122 | 186 | 34.5 | 24 | 3.13 | 665 | 152 | 232 | 34.5 |
| 13 | 2.89 | 522 | 105 | 192 | 45.0 | 2.78 | 497 | 105 | 173 | 39.0 | 6.0 | Mild | 8 | 2.89 | 722 | 99 | 152 | 35.5 | 11 | 2.95 | 547 | 98 | 146 | 33.0 |
| 14 | 2.27 | 450 | 92 | 155 | 41.0 | 2.27 | 487 | 113 | 185 | 39.0 | 3.0 | Mild | 13 | 2.38 | 550 | 118 | 174 | 32.0 | 16 | 2.44 | 600 | 129 | 187 | 31.0 |
| 15 | 3.58 | 582 | 137 | 201 | 32.0 | 3.52 | 590 | 160 | 232 | 31.0 | 7.5‡ | Severe | 15 | 3.46 | 668 | 129 | 177 | 27.0 | 17 | 3.40 | 606 | 123 | 185 | 33.5 |
| 16 | 2.84 | 543 | 112 | 200 | 43.5 | 3.07 | 574 | 125 | 208 | 40.0 | 6.0‡ | Severe | 15 | 3.01 | 700 | 127 | 199 | 36.0 | 17 | 3.07 | 603 | 124 | 200 | 38.0 |
| $\bar{x}$ | 2.72 | 526 | 117 | 197 | 40.8 | 2.73 | 542 | 125 | 198 | 37.0 | 5.1 | | 14.6 | 2.80 | 654 | 118 | 181 | 34.9 | 17.5 | 2.85 | 599 | 128 | 200 | 36.2 |
| s | 0.49 | 80.7 | 14.7 | 22.2 | 3.6 | 0.45 | 91.6 | 19.5 | 24.0 | 3.3 | | | | 0.42 | 85.9 | 9.6 | 14.6 | 3.6 | | 0.41 | 73.4 | 22.2 | 34.4 | 3.3 |
| $s_{\bar{x}}$ | 0.15 | 25.5 | 4.7 | 7.0 | 1.1 | 0.14 | 29.0 | 6.2 | 7.6 | 1.1 | | | | 0.13 | 27.1 | 3.0 | 4.6 | 1.1 | | 0.13 | 23.2 | 7.0 | 10.9 | 1.0 |

* The *first response* is that immediately preceding the detection of antibodies; the second response is that on the day precipitins appeared.

† The "Day" indicated is the time after the first feeding.

‡ The dose was given in two equal feedings.

After a baseline for the individual animal had been established, the 10 rabbits in group 2 were each forced to eat 3 to 7.5 Gm. of the trichinous rat meat (Table I). Five of the animals were fed half of the infecting dose on each of two successive days. All of the animals in group 2 were followed at least 30 days following the feeding of trichinous meat, unless accidental death from hemopericardium supervened.

*Statistical analysis:* The details of the statistical methods have been previously described (2). The total blood volume, plasma volume, hematocrit and thiocyanate space were recorded in absolute values (Table I). The mean of two successive baseline determinations on each animal was compared with the mean of two successive determinations made just before the precipitin test became positive and on the day precipitins appeared. In this fashion, each animal in the experimental group (group 2) served as its own control.

The data as obtained in absolute values were then calculated in terms of milliliters per kilogram of body weight on the day the measurement was made.

The data were further analyzed comparing the first baseline determination with the determination made just prior to the appearance of precipitins, and comparing the first baseline determination with the determination made on the day precipitins were first detected. These response values were also analyzed in terms of absolute values and of milliliters per kilogram of initial body weight.

## RESULTS

*Control on methods:* The data in the uninfected animals (group 1) are in accord with those previously published and are not presented in detail since they are not utilized in the statistical calculations in the infected animals (group 2) (2, 3).

*General observations:* None of the animals in group 1 showed any symptoms or signs suggesting trichinosis. No reaction was noted in any rabbit to the repeated intravenous injection of dye and no evidence of sensitization was detected by skin or precipitin test.

Clinical evidence of infection appeared in seven of 10 animals in group 2 within the first 10 days following the feeding of trichinous rat meat. In five of these animals the skin became soggy, hot, and moist between the fifth and ninth days and remained so for a maximum of five days. This edema was most striking in the skin over the abdomen but was not evident unless the fur had been clipped. In the other two animals the initial sign of infection was the appearance of a thin, hot, dry skin between the eighth and 10th days. In these seven animals the clinical signs reached a maximum and largely subsided before the precipitin test became positive.

Three animals developed clinical signs after the 10th day of feeding. In one the skin became hot and dry on the 15th day—the time at which a positive precipitin test was first detected. Another rabbit developed edema after the appearance of a positive precipitin test.

In general the clinical severity of early trichinosis appeared to be directly related to the number of muscle trichinae ingested (Table I). The rabbit fed the largest amount of trichinous meat (7.5 Gm.) developed the severest clinical edema. The three animals in which clinical signs appeared late in the course of observation had been fed the minimal dose of rat meat (3 Gm.). In general the changes in the distribution of body fluid measured paralleled the clinical severity of the disease process.

At the conclusion of the experiment a sample of meat from each rabbit was examined by either the muscle press or digestion technique; all the animals in group 2 were infected.

Four of the animals appeared jaundiced; one on the fifth, two on the 10th, and one on the 11th day after feeding.

*Immunologic findings:* In all 10 animals in group 2 precipitin tests to trichinella antigen became positive and remained so during the period of observation; the distribution was as follows:

| | Onset of positive precipitin tests | | | | | | |
|---|---|---|---|---|---|---|---|
| Days after feeding | 11 | 15 | 16 | 17 | 19 | 20 | 24 |
| Number of rabbits | 1 | 1 | 1 | 3 | 2 | 1 | 1 |

*Thiocyanate space:* Increases in the thiocyanate space developed at some time during the period of observation in all of the animals in group 2 (Table I). In all except two the increase in thiocyanate space had begun to subside before the precipitin test became positive. In six animals the thiocyanate space increased a second time following the development of a positive precipitin test (Figure 1).

The alteration in thiocyanate space did not parallel the changes in body weight. At some time during the period of observation, in all animals, the body weight did not change appreciably or increase as the thiocyanate space increased. As the thiocyanate space subsequently decreased, this alteration was again out of proportion to the change

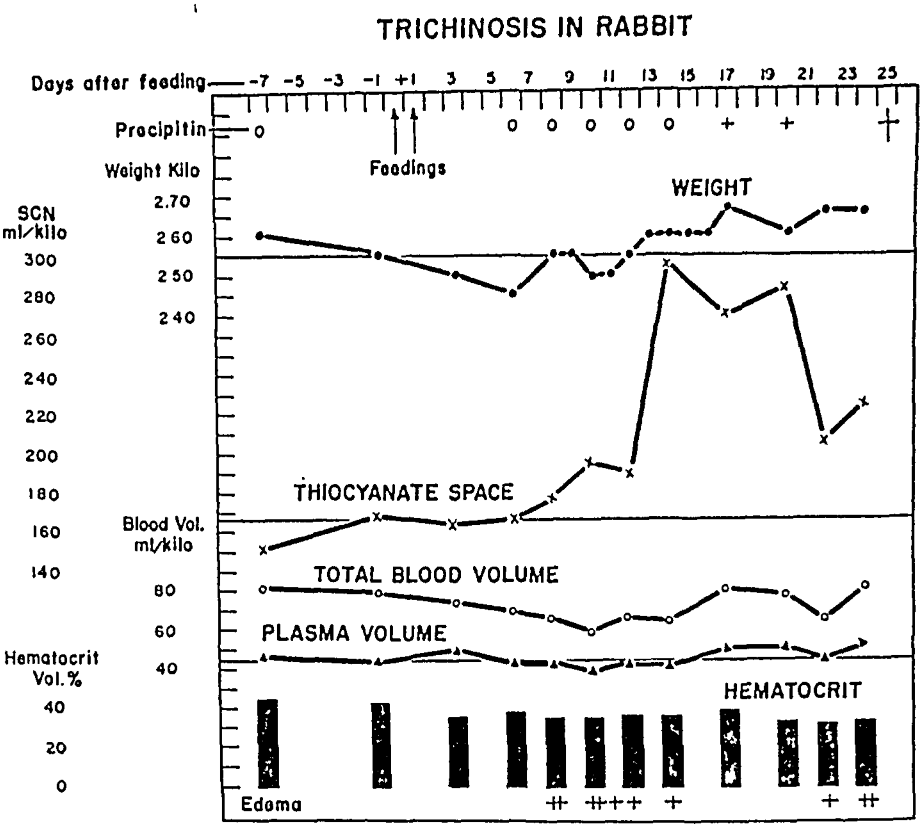

Fig. 1. Fluid Changes in a Rabbit Fed 6 Gm. of Trichinous Rat Meat

Note the increase in thiocyanate space on the 14th day after the first feeding, without a concomitant relative increase in body weight. The blood volume was decreased slightly during the 10th to 14th days. This rabbit was severely ill; it developed clinical jaundice on the 10th day. The physiological changes were reversing when the precipitin became positive on the 17th day.

in body weight and could not be accounted for on this basis alone.

When the mean of the two baseline determinations is compared statistically with the mean of the two response values recorded in the table, the value of $\bar{d}$ is found to be + 90.6; $s_{\bar{d}}$ 29.1; t 3.11, giving a value for P between 0.01 and 0.02. When the analysis is performed utilizing the first baseline and the data obtained just *before* the appearance of the positive precipitin test, the data are highly significant whether analyzed in terms of absolute values or of milliliters per kilogram of weight on that day. The corresponding figures are, respectively: $\bar{d}$ + 128.5, $s_{\bar{d}}$ 29.4, t 4.37, P less than 0.01; and $\bar{d}$ + 39.8, $s_{\bar{d}}$ 11.8, t 3.37, P less than 0.01. When the first baseline value is compared with the first response in terms of milliliters per kilogram of initial (baseline) body weight, the figures are also highly significant with t 4.25 and P less than 0.01.

*Blood volume:* Alterations in the plasma volume were not significant by any means of calculation. When alterations in the total blood volume were calculated in terms of milliliters per kilogram of body weight on the day of the first response and were compared with the first baseline determination, the value of t was 3.25 with P 0.01. No other values were significant.

On the whole the minor decreases in blood volume tended to precede the development of a positive precipitin test and paralleled the clinical severity of the disease.

*Hematocrit:* A steady decrease in hematocrit was observed early in the experiment and tended to disappear with recovery. The values were statistically significant with P less than 0.01 when the first baseline value was compared with either the first or second response value. The t values were respectively 6.13, 3.55.

*Weight:* The changes in weight were not considered statistically significant since the value for P was always greater than 0.05.

## DISCUSSION

The experiments indicate that rabbits experimentally infected with trichinosis exhibit alterations in the fluid space which is available for the diffusion of thiocyanate ion. The maximal alterations occur just before the appearance of circulating humoral antibodies (precipitins) in the serum. This finding correlates well with the previous clinical observation that the symptoms of serum sickness begin to disappear with the appearance of precipitins (6).

In trichinosis, however, the immunologic problem is more complicated than in serum sickness. Serum disease is usually produced by a single injection, containing all antigens, and the response is dramatic. In trichinosis, on the other hand, multiple antigens are being continuously introduced over a period of weeks. The two increases in the thiocyanate space detected in some animals may be the result of antibody responses to two distinct antigens. Differences have been suspected in the antigens contained in the bodies of adult and larval forms of trichinae (7); it is possible that the antigenic components of immature, unencysted trichinella larvae may also be slightly different from the mature encysted forms. Previous studies have demonstrated that peptic and tryptic digestion destroys the protein fraction of the larvae, but does not destroy the antigenicity of some remaining component which presumably is a carbohydrate (8). The adult female attaches to the intestinal mucosa and secretes an antigen which probably is of little significance in the acute disease; the antibody, however, is concerned with the prevention of re-infection. Because of the relatively small number of adults in relation to the huge number of larvae, it is likely that the larval antigens are of greater concern in acute trichinosis. In any event, the physiologic alterations caused by different antigenic substances appear to vary quantitatively but not qualitatively.

*Permeability of capillary membranes:* Observations on human beings with rickettsial spotted fever and on rabbits with experimental serum sickness have been interpreted as indicating that alterations in the permeability of the vascular tree occurred (1, 2). The degree of alteration varied with the clinical severity of the disease process. In mild instances water and ions which normally traverse the capillary membrane would leave the blood more readily. Larger molecules such as protein would be lost in more severe instances and red blood cells would pass out of the vascular tree only in the most severe reactions.

During the stage of edema of the muscles in severe clinical trichinosis the accumulation of fluid is associated with oliguria which lasts from one to two weeks and is followed by diuresis. This sequence of events is also observed during pneumonia and serum disease and is accompanied by retention of water, sodium and chloride (9, 10). These clinical findings can be explained by an increase in capillary permeability; water and ions lost from the vascular tree must be retained in the tissues.

When serial determinations of the blood proteins have been made in clinically severe instances of the disease hypoproteinemia has been found (11). The minimal decreases in blood volume detected by the dye method indicate that in the experiments reported the alteration in capillary permeability to molecules larger than those of salt and water was equivocal. Rough determinations of the serum proteins by a microtechnique showed only slight alterations in these rabbits. Protein levels in the plasma and interstitial fluid of patients with the disease should be followed serially, especially during the stage of edema, to determine whether proteins leak into interstitial spaces. Information on the rate of protein excretion is also needed to estimate the degree of tissue destruction and of protein loss.

The drop in the hematocrit without evidence of intravascular hydration indicates that red blood cells were lost from the vascular compartment. The drop was apparently not due to the repeated cardiac punctures since the hematocrit remained constant in group 1 and other experiments have shown that the amount of blood withdrawn is inadequate to produce this change (2, 3). No petechiae were noted and it is unlikely that the degree of hemorrhage which accompanies the breaking out of larvae from capillaries into interstitial muscle spaces is adequate to explain the drop in hematocrit. It is known that the trauma which occurs during penetration of gravid trichinae into the mucosa of the small intestine results in microscopic ulceration and bleeding. No evidence has been presented, however, that intestinal trichinae ingest red blood cells in the manner that the hookworm does. The icteric tint of the serum and clinical jaundice observed in four rabbits during the period when the blood volume was reduced suggest that some red cells may have been hemolyzed. Intravascular hemolysis results from antigen-antibody reactions which directly involve red blood cells, but no such process is recognized in trichinosis.

*Cellular permeability:* It is not known whether the thiocyanate ion normally diffuses into cells or not. The fact that some rabbits showed an increase in thiocyanate space without a comparable increase in body weight would favor the thesis that some re-distribution of fluid had occurred, whether or not additional fluid ingested in food or water was retained. It would appear that the increase in thiocyanate space is not due entirely to an increase in fluid between cells but is indicative of increased hydration within cells. If this be true, the reversible psychic disturbances seen during the stage of edema in acute clinical trichinosis may be due to excessive hydration of nerve cells.

The appearance of positive skin tests in patients with trichinosis has been interpreted as indicating the development in the disease of cellular immunity and hypersensitivity which often is of marked degree. It is likely that the cellular component of immunity of trichinosis is greater than the humoral component. If the appearance of circulating antibodies is indicative of prior neutralization of antigens attached to cells as well as circulating in the blood, the maximum physiologic alterations resulting from their interaction would precede the detection of humoral immunity. Such was the case. Apparently an antigen-antibody reaction occurred either on the cell surface (as probably occurs in serum sickness) or within the cell (as in rickettsial disease); the cell was injured and the permeability of the cell membrane to the thiocyanate ion was increased.

*Mechanisms:* It has been postulated that histamine or some similar substance is released during the interaction of antigens with antibodies. The liberation at the cell or capillary wall of some substance which would increase the permeability of the cell membranes would enhance the transmission of molecules including the thiocyanate ion across the surface. It is not known whether the antigens in trichinosis attach to the vascular wall or to tissue cells outside the vascular system as well. The anatomic location of trichinella larvae in the interstitial spaces would favor the latter hypothesis.

In the microscopic examination of human muscle, when no trichinae are found it is sometimes difficult to differentiate trichinosis from periarteritis nodosa, and a possible relationship between the two diseases has been suggested (12). Lesions resembling periarteritis have been found in rabbits sensitized to horse serum (13). The above observations add further support to the view that the symptoms and signs of clinical trichinosis may be at least partially due to sensitization of the host to antigenic substances contained in or secreted by trichinae.

The present study indicates that the physiologic alterations which may result from antigen-antibody reactions occurring during an interstitial infection are more profound than has been appreciated in the past. The principles of supportive therapy previously described in Rocky Mountain spotted fever which emphasize alterations in permeability of membranes may be usefully applied to the treatment of patients with trichinosis (14). The possibility that the administration of antihistaminic drugs may decrease the physiologic alterations resulting from the immune or allergic reactions should be considered.

## SUMMARY

1. Trichinosis, an extracellular infection, is accompanied in rabbits by clinical edema and an increase in the fluid space available for dilution of thiocyanate ions.

2. The changes were interpreted as indicating an increase in permeability of the vascular tree, probably in the capillary wall, so that crystalloids were lost from the circulation into the interstitial spaces.

3. Occasional discrepancies observed between the increase in thiocyanate space and the changes in weight suggest that cellular permeability is probably also increased to water and ions.

4. A significant drop in hematocrit occurred; in occasional animals it was accompanied by clinical jaundice and the appearance of icterus in the serum.

5. The physiologic changes were maximal just before humoral antibodies( precipitins) appeared, suggesting that the alterations may have been due

directly or indirectly to an antigen-antibody reaction.

6. The clinical symptoms and signs of trichinosis are not solely due to mechanical blockage of capillaries or arterioles by migrating larvae, but are more likely the result of re-distribution of fluid in the extravascular spaces.

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# BACTERIAL CAPSULAR POLYSACCHARIDES IN THE CEREBROSPINAL FLUID OF PATIENTS WITH PURULENT MENINGITIS[1]

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(Submitted for publication February 12, 1951; accepted, March 19, 1951)

With increasing use of antibiotics, the recovery of specific causative organisms in bacterial infections is made less frequently. Failure to demonstrate the specific bacteriological agent involved is commonly encountered in patients hospitalized after the administration of varying amounts of antibiotics. During the course of infections due to the pneumococcus, type-specific capsular polysaccharides have been detected in blood, urine, sputum and pleural exudate (1–6). There is no record of the demonstration of type-specific capsular polysaccharides in the cerebrospinal fluid of patients with pneumococcic meningitis, although the occurrence of capsular polysaccharides in the cerebrospinal fluid of patients with meningococcic meningitis is well established (7–10). The purpose of this communication is to report the detection of capsular polysaccharides in the cerebrospinal fluid of five patients with pneumococcic meningitis and one patient with meningitis due to Klebsiella pneumoniae.

## MATERIALS AND METHODS

Group and type-specific rabbit anti-pneumococcal sera, types 1 to 33, were obtained from the New York City Department of Health. Group-specific rabbit anti-Klebsiella pneumoniae sera, groups A and B, were provided by Dr. Michael Heidelberger.

As has been previously shown, the presence of C-polysaccharide and C-antibody may interfere with the interpretation of positive precipitin reactions (7, 8). In the present studies, the cerebrospinal fluid of two patients with pneumococcic meningitis showed the presence of C-polysaccharide when tested with horse-serum containing a high titre of C-antibody. For that reason, all typing and grouping sera used were first absorbed with 5 micrograms of C-polysaccharide per milliliter of serum to remove the C-antibody present. The C-polysaccharide used in the absorption was derived from a type VII pneumococcus.

In attempting to demonstrate the presence of a pneumococcus polysaccharide, a preliminary test was done using polyvalent sera, types 1 to 33, each containing precipitins to four types of pneumococci. Five-tenth milliliter portions of C-absorbed group-specific antisera were placed in small sterile test tubes. Five-tenth milliliter portions of cerebrospinal fluid, which had been cleared by centrifugation, were added to each of the tubes containing group-specific antisera. Saline was added to the serum and cerebrospinal fluid control specimens. The contents of the tubes were mixed and allowed to stand for one hour at room temperature. The tubes were then centrifuged and examined for the presence of a precipitate; if none was visible, the tubes were placed at 4° C. and reexamined at the end of 24 and 48 hours. If one of the tubes contained a precipitate, the cerebrospinal fluid was then set up in a similar manner against each of the four type-specific antisera in that group.

When attempting to demonstrate the presence of capsular polysaccharide of Klebsiella pneumoniae, in addition to tests with specific antisera, one spinal fluid sample was set up against type II pneumococcus antiserum. It has been demonstrated that the capsular polysaccharide of Klebsiella pneumoniae group B organisms cross-reacts with antiserum to type II pneumococcus (11).

## RESULTS

Seventeen specimens of cerebrospinal fluid from 10 patients with purulent meningitis were tested. All of the patients received penicillin in large dosage by parenteral and intrathecal routes immediately after the initial cultures of the cerebrospinal fluid were obtained. Results of cultures and the precipitin tests are shown in Table I. All control tests were negative. Specimens from two patients showing no growth on culture were negative for pneumococcal polysaccharide. One patient with Actinomyces bovis and one patient with Cryptococcus hominis on spinal fluid culture showed no pneumococcus polysaccharide. One patient from whom an untypable Friedlander's bacillus was cultured, was shown to have capsular polysaccharide of Klebsiella pneumoniae group A in his spinal fluid. The corresponding type-specific capsular polysaccharide was demonstrated in the spinal fluid of each patient with proven pneumococcic meningitis. One patient in whom a pneumococcus infection was suspected but with a negative spinal fluid culture showed type I capsular polysaccharide.

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[1] This study has been aided by a grant from the American Cancer Society.

[2] John and Mary R. Markle Scholar.

TABLE I

| Patient | Culture of CSF | Test for polysaccharide in CSF | Persistence of polysaccharide |
|---|---|---|---|
| | | | (days) |
| 1 | Negative | Negative | — |
| 2 | Negative | Negative | — |
| 3 | Actinomyces | Negative | — |
| 4 | Cryptococcus hominus | Negative | — |
| 5 | Kleb. pneum. ? type | Kleb. pneum. Group A | 3 |
| 6 | Pn. VIII | Pn. VIII | 3 |
| 7 | Pn. VI | Pn. VI | 6 |
| 8 | Pn. XIX | Pn. XIX | 2 |
| 9 | Pn. VI | Pn. VI | 8 |
| 10 | Negative | Pn. I | — |

The polysaccharides, when detected, were shown to persist in specimens obtained at least two days after the cerebrospinal fluid cultures were negative. In one patient, the polysaccharide was still demonstrable eight days after the cultures had become negative (see Table I).

## COMMENT

The test described is quite sensitive since as little as 2 or 3 micrograms of capsular polysaccharide, in the presence of a potent specific antiserum, will give an easily visible precipitate. A positive test is probably specific if preliminary removal of C-antibody from the typing sera is carried out. A negative test does not necessarily rule out the possibility of a pneumococcal meningitis because, under the conditions of the test, there is no information on the absolute amount of antigen added. Cerebrospinal fluid containing capsular polysaccharide in low concentration may have to be added in large amounts to produce a visible precipitin reaction. In the presence of unusually high concentrations of capsular polysaccharide, the precipitin reaction may possibly be inhibited by virtue of entering the zone of large antigen excess (*12*).

Identification of the specific causative organism in bacterial meningitis has been made much more difficult because of the liberal use of antibiotics. The detection of bacterial polysaccharides in the cerebrospinal fluid may be of assistance in making the specific bacteriological diagnosis in patients with meningitis who have received previous antibiotic therapy.

## SUMMARY

1. Capsular polysaccharides have been demonstrated in the cerebrospinal fluid of five patients with pneumococcic meningitis and one patient with meningitis due to Klebsiella pneumoniae.

2. The capsular polysaccharide was still demonstrable in cerebrospinal fluid specimens taken from two to eight days after the cerebrospinal fluid cultures showed no growth.

3. The demonstration of specific capsular polysaccharide in the spinal fluid may be of diagnostic value in establishing the nature of the pathogen involved in patients in whom the organism cannot be recovered because of previous antibiotic treatment.

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# DIETARY TREATMENT OF HYPERTENSION. II. SODIUM DEPLETION AS RELATED TO THE THERAPEUTIC EFFECT[1]

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(Submitted for publication October 31, 1950; accepted, March 26, 1951)

Abnormal accumulations of sodium have been demonstrated by direct analysis of the tissues of dogs (1) and rats (2) that had been made hypertensive by renal ischemia. These observations are of special interest because of the reductions of blood pressure in hypertensive patients that may be induced by means of extreme restriction of the dietary sodium. Taken together, these facts suggest that the benefit experienced by the patients might be due to the removal of excess sodium.

On the other hand, it must be recognized that hypertension is not a necessary, or even a usual, consequence of sodium retention. Accumulations of extracellular fluid in association with normal levels of blood pressure are of frequent occurrence in a variety of diseases, including renal disorders. Therefore, if the retention of sodium is to be regarded as contributory, rather than as merely incidental, to the development of hypertension, it must be linked to some other metabolic disturbance in this particular disease.

In the present study five hypertensive patients were subjected to large changes in their rations of dietary sodium. Resultant changes in the sodium content of their bodies were measured by isotope dilution. These observations have been correlated with the changes in their clinical status and with inferred changes in the degree of cellular hydration. It is suggested that the latter may be the important factor that is linked to the depletion of sodium.

## PROCEDURE

Five patients with benign and uncomplicated hypertension were chosen for a study. Apart from Grade I changes in their ocular fundi and slight enlargements of the heart shadow in their chest films, the results of physical and laboratory examinations were negative. In particular all patients had normal urea clearances and absence from the urine of significant quantities of protein, casts and cells. Further clinical data are summarized in Table I; similar data also are given for the three normal subjects who served as controls in the measurement of exchangeable sodium.

During the five to seven months of this study the patients remained entirely on the metabolic ward, except for a vacation of three days during the Christmas season. Confinement to a hospital ward necessarily limited the physical activity of each patient; at no time, however, were the patients restricted to bed.

A central feature in the design of the investigation was the provision of an extended time during which the sodium intake was maintained at a high normal value (180

TABLE I

*Clinical data*

| Patient | R. I. H. No. | Age | Sex | Family history of hypertension | Known duration of hypertension | Disease other than hypertension | Ratio initial wt. / ideal wt.* |
|---|---|---|---|---|---|---|---|
| | | *years* | | | *years* | | |
| A | 12,450 | 26 | female | +++ | 4 | 0 | 1.12 |
| B | 12,447 | 44 | female | 0 | 7 | 0 | 1.07 |
| C | 12,451 | 27 | male | + | 5 | migraine | 1.12 |
| D | 12,453 | 39 | female | 0 | 19 | 0 | 1.06 |
| E | 12,465 | 41 | male | 0 | 10 | 0 | 1.12 |
| F | normal control | 35 | male | 0 | 0 | 0 | 1.01 |
| G | normal control | 37 | male | 0 | 0 | 0 | 1.06 |
| H | normal control | 28 | male | + | 0 | 0 | 0.85 |

* "Ideal weight" for each patient was calculated from normal values which had been derived from life insurance statistics (7).

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[1] The radiosodium used in this investigation was supplied by Oak Ridge on allocation from the Isotopes Division, United States Atomic Energy Commission.

meq./day) by the addition of salt tablets to a diet otherwise low in sodium (less than 7 meq./day). During this preliminary time, changes in the daily intakes of water and of protein were imposed in order to demonstrate any clinical effects of these factors in the absence of sodium restriction. After from two to four months had elapsed, the intake of sodium was reduced to the 7 meq./day level by omission of the supplementary tablets. Ten weeks later the sodium intake was returned to the higher level by resumption of the supplement in order to test the reversibility of the clinical and metabolic changes which had followed the restriction of this element.

The detailed timing of these manipulations may be seen in the figures. The total study was divided into six periods, which are separated in all the figures by vertical lines. These periods were differentiated by changes in the water or the sodium intake; one of these two factors remained constant throughout any consecutive pair of periods while the other was changed. An upward direction of the arrow in relation to the symbols in the figures indicates that this factor was administered in high dosage during the period; a downward direction indicates the converse. For sodium the high dosage level was 180 meq./day and the low dosage was the residual amount in the diet alone, namely a value less than 7 meq./day. During the periods of high water intake, 4,500 cc./day of fluid were administered in addition to the preformed and metabolic water of the diet; as a result the urinary volumes were maintained at high average values (Table II). Water restriction in the low dosage periods was carried to the point of slight thirst; an intake of 800 to 1,000 cc./day of supplementary fluid, which maintained the urinary volumes in the range 600 to 900 cc./day, appeared to be the minimum tolerable fluid supplement when sodium was concurrently administered in high dosage. In the figures a symbol for water in Period I is absent because the patients were allowed uncontrolled access to water; during this time their urine volumes ranged between 800 and 1,800 cc./day.

The changes in level of protein intake are indicated in Figure 3. It may be seen that the daily consumption was 30–40 grams until the middle of Period III, when the amount was approximately doubled. Prior to this time the patients had felt dissatisfied with the diet despite the fact that it was as diversified as was compatible with the requirement of sodium restriction, and was unlimited in quantity except with respect to the foods of moderate sodium content. Since the usual high protein foods fell into the latter category, the protein intake was curtailed until Period III, when a special low sodium, high protein food[2] was made available for the patients to take in amounts regulated by their appetites. During Period IV they continued to take the increased amount of protein; with the restriction of water in Periods V and VI the amount of this supplement that could be conveniently given was limited by the lack of fluid for its suspension.

One exception to the plan of deferred sodium restriction should be noted: through oversight, the supplementary salt tablets were not ordered for Patient C. When the omission was discovered he had already shown a reduction in blood pressure below the value on admission and the earlier values that had been observed in the out-patient clinic. Therefore, it was decided to continue the study of his response without the supplement until the final period.

## METHODS

Daily measurements of blood pressure and of body weight were made each morning except Sundays under standardized conditions. The blood pressure was determined by the same physician for each patient; at least 20 minutes of bed rest preceded the reading. Body weight was measured after urination and before breakfast on a calibrated scale that was accurate to 100 grams; the patients were nude except for a covering sheet of known weight.

At biweekly intervals a standard 2 meter chest film was obtained for measurement of heart size. At monthly intervals the basal metabolic rates were measured, and electrocardiographic tracings taken.

A total collection of urine was made, and divided into 24 hour pools; analyses of sodium, potassium and chloride concentrations were made upon each, using methods previously discussed (3). During two intervals of five days each, at the ends of Periods IV and VI, collections of stools were made; the total output from each interval was pooled and analyzed for sodium and potassium (3).

The determination of exchangeable sodium was based on the dilution of a measured dose of radiosodium. An infusion fluid containing $0.8-1.0\,\mu c$ of $Na^{24}$ Cl/cc. of 5 per cent dextrose in water was injected intravenously from a calibrated 100 cc. burette at a dose of just 1 cc./kg.[3] Samples of blood were taken at hourly intervals for seven hours; in some tests additional samples were taken after 24 and 48 hours.

With a Tracerlab $\beta$-$\gamma$ dip-counter (TGC-5) and autoscaler, measurements of the radioactivity of the samples were made after dilutions that were sufficient to bring the counting rates within the range of linear response of the tube. An equilibrium value (a) of the specific activity of the serum (ratio of radioactivity in arbitrary units to the sodium concentration) as attained in the five to seven hour interval was estimated graphically. The quantity (q) of sodium with which the injected dose (d) of tracer had been mixed was calculated from the relation $q = d/a$. This quantity is about 10 per cent less than that which would be calculated from the 24 hour equilibration of specific activity and therefore does not include the

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[2] "Low sodium food," Wyeth: protein, 50 per cent; carbohydrate, 19.5 per cent; fat, 25 per cent; total ash, 3.5 per cent; sodium, 0.02 per cent. A generous supply of this product was donated by Wyeth, Inc.

[3] Calculation based on the data given by Marinelli and associates (4) indicated that the patients were exposed by this test to less than 0.1 equivalent roentgen of radiation during the first 24 hours and to negligible amounts thereafter.

TABLE II—*Measurements of clinical status and body sodium content*

| Subject | Period | | Average sodium intake | Average urine volume | Blood pressure means (and standard deviation) | | Heart size | | Exchangeable sodium measurements | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | Time of measurement | | Body weight | | Sodium | |
| | No. | Duration | | | Systolic | Diastolic | Maximum diameter | Internal diameter of chest | After sodium restriction | After sodium increase | Actual wt. | Actual wt./Ideal wt. | Per kg. | Total |
| | | *days* | *meq./day* | *cc./day* | *mm. Hg* | *mm. Hg* | *mm.* | *mm.* | *days* | *days* | *kg.* | | *meq./kg.* | *meq.* |
| A | I | 36 | 180 | 780 | 168 (7) | 108 (11) | 141 | 272 | | | 76.2 | 1.12 | | |
| | II | 47 | 180 | 660 | 169 (10) | 109 (7) | 138 | 271 | | | 70.9 | 1.04 | | |
| | III | 34 | 180 | 3660 | 170 (4) | 106 (4) | 130 | 272 | −1 | | 71.3 | 1.05 | 39.9 | 2850 |
| | IV | 45 | 7 | 3770 | * | * | * | | 28 | | 70.0 | 1.03 | 39.0 | 2730 |
| | | | | | 136 (7) | 81 (7) | 115 | 268 | 43 | | 70.2 | 1.03 | * 34.8 | * 2440 |
| | V | 24 | 7 | 900 | 131 (6) | * 73 (8) | 118 | 272 | 64 | −5 | 69.4 | 1.02 | 35.7 | 2480 |
| | VI | 18 | 180 | 1160 | * 158 (6) | * 96 (2) | * 138 | 272 | | 16 | 70.2 | 1.03 | * 40.4 | * 2830 |
| B | I | 41 | 180 | 800 | 233 (13) | 132 (8) | 139 | 260 | | | 60.7 | 1.07 | | |
| | II | 47 | 180 | 785 | 233 (17) | 131 (11) | 139 | 263 | | | 57.0 | 1.00 | | |
| | III | 34 | 180 | 3775 | 227 (15) | 127 (8) | 139 | 258 | −1 | | 57.6 | 1.01 | 38.1 | 2195 |
| | IV | 45 | 7 | 3720 | * 188 (15) | * 110 (7) | 132 | 258 | 28<br>43 | | 56.6<br>56.6 | 0.99<br>0.99 | * 31.7<br>32.1 | * 1826<br>1817 |
| | V | 24 | 7 | 3835 | 184 (14) | * 95 (9) | 135 | 262 | 64 | −5 | 55.8 | 0.98 | 33.8 | 1886 |
| | VI | 18 | 180 | 4000 | * 220 (18) | * 116 (7) | 140 | 257 | | 16 | 55.7 | 0.98 | * 42.4 | * 2362 |
| C | I | 35 | 7 | 590 | 145 (12) | 83 (8) | 146 | 298 | | | 69.1 | 1.12 | | |
| | II | 47 | 7 | 430 | 140 (15) | 85 (7) | 142 | 295 | | | 66.2 | 1.07 | | |
| | III | 34 | 7 | 3050 | 141 (11) | 88 (8) | 139 | 285 | 119 | | 65.0 | 1.05 | 34.3 | 2230 |
| | IV | 44 | 7 | 3890 | 142 (7) | 87 (6) | 146 | 289 | 147<br>162 | | 63.7<br>64.4 | 1.03<br>1.04 | 33.6<br>32.5 | 2140<br>2093 |
| | V | 25 | 7 | 810 | 140 (9) | * 78 (9) | 140 | 295 | 183 | −5 | 63.7 | 1.03 | 35.3 | 2249 |
| | VI | 18 | 180 | 990 | 148 (3) | * 91 (4) | * 150 | 295 | | 16 | 65.1 | 1.05 | * 39.6 | * 2578 |

TABLE II—*Continued*

| Subject | Period | | Average sodium intake | Average urine volume | Blood pressure means (and standard deviation) | | Heart size | | Exchangeable sodium measurements | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | Time of measurement | | Body weight | | Sodium | |
| | No. | Duration | | | Systolic | Diastolic | Maximum diameter | Internal diameter of chest | After sodium restriction | After sodium increase | Actual wt. | Actual wt. / Ideal wt. | Per kg. | Total |
| | | *days* | *meq./day* | *cc./day* | *mm. Hg* | *mm. Hg* | *mm.* | *mm.* | *days* | *days* | *kg.* | | *meq./kg.* | *meq.* |
| D | I | 26 | 180 | 1585 | 177 (11) | 108 (11) | 139 | 293 | | | 69.7 | 1.06 | | |
| | II | 47 | 180 | 665 | 173 (9) | 103 (9) | 142 | 285 | | | 64.9 | 0.99 | | |
| | III | 34 | 180 | 3960 | 170 (9) | 103 (8) | 142 | 290 | −1 | | 65.2 | 1.00 | 38.1 | 2484 |
| | IV | 45 | 7 | 3940 | *<br>145 (6) | *<br>90 (6) | 135 | 288 | 28<br>43 | | 63.2<br>62.8 | 0.97<br>0.96 | *<br>32.6<br>31.8 | *<br>2060<br>1997 |
| | V | 24 | 7 | 860 | 140 (8) | 88 (8) | *<br>125 | 287 | 64 | −5 | 61.7 | 0.94 | 35.3 | 2178 |
| | VI | 18 | 180 | 1255 | 150 (12) | 93 (10) | *<br>138 | 281 | | 16 | 62.6 | 0.96 | *<br>39.6 | *<br>2479 |
| E | I | | | | | | | | | | | | | |
| | II | 26 | 180 | 930 | 204 (15) | 111 (10) | 165 | 338 | | | 92.6 | 1.12 | | |
| | III | 34 | 180 | 3670 | 215 (9) | 114 (11) | 166 | 328 | −1 | | 92.0 | 1.11 | 44.5 | 4094 |
| | IV | 45 | 7 | 3155 | 213 (15) | 105 (10) | 162 | 329 | 28<br>43 | | 90.5<br>90.6 | 1.09<br>1.09 | *<br>35.8<br>36.5 | *<br>3239<br>3307 |
| | V | 24 | 7 | 470 | 206 (23) | *<br>93 (9) | 169 | 331 | 64 | | 88.2 | 1.06 | 38.8 | 3422 |
| | VI | 18 | 7 | 430 | 218 (20) | 88 (8) | 163 | 329 | 75 | | 87.7 | 1.06 | 39.3 | 3447 |
| F | Normal controls | | | | | | | | | | 77.2 | 1.01 | 35.3 | 2725 |
| G | | | | | | | | | | | 82.7 | 1.06 | 35.0 | 2894 |
| H | | | | | | | | | | | 57.7 | 0.85 | 40.7 | 2348 |

* Asterisk indicates a significant difference between consecutive values.

most slowly equilibrating sodium in the body. It is to be expected, however, that equilibration would have been complete in the soft tissues which have been shown (1, 2) to accumulate sodium in the animals with hypertension.

### *Criteria of Significance*

Average values of blood pressure were calculated from the consecutive readings obtained during the last two weeks of each period except Period VI for which only the last week was taken. Fisher's "t test" (5) was used to calculate the probability that the differences between successive averages might occur by chance alone. Changes were considered significant when this probability was less than 0.01; these changes are indicated by an asterisk in Table II.

Heart diameter on a given chest film could be measured reproducibly within 2 mm. In order to exclude the effects of differences in projection and heart cycle it was taken as necessary that changes must exceed 10 mm. to be significant. These are indicated in Table II.

The exchangeable sodium is calculated from four primary measurements: the counting rates of solution injected and of the serum at equilibrium, the volume of fluid injected and the sodium concentration of the serum sample. Since the fluid was delivered into the subject from a calibrated burette which was accurate to less than 0.1 cc., the error in this factor may be neglected. The average counting rates of the diluted infusion fluid, diluted serum and the background were 5,000, 400, and 44 cpm., respectively, and were counted for 20,480, 2,048, and 4,096 total counts. From these values it may be estimated that the coefficients of variation (ratio of standard deviation to mean value) were 0.007 and 0.022, respectively, for the infusion fluid and the serum samples (6). For the variation in the determination of total sodium concentration the coefficient 0.020 was assumed. These factors contribute to the uncertainty of estimation of their product according to the formula $E = (F^2 + S^2 + C^2)^{1/2}$ where the symbols indicate the coefficients of variation of the exchangeable sodium, the infusion fluid counting rate, the serum counting rate, and the serum sodium concentration, respectively. Substitution of the values above give $E = 0.031$. Since the final value for body sodium was based on at least three independent serum samples the coefficient for the final result is reduced by the factor $(1/\sqrt{3})$ and thus was approximately 0.02. In the case of an adult of average weight this value corresponds to a standard deviation of less than 60 meq. The changes discussed in this paper were of considerably greater magnitude; in Table II, under the heading of "Exchangeable sodium," the asterisks signify a change of five standard deviations or more.

## OBSERVATIONS

The blood pressures of all patients remained elevated during the periods in which the intake of sodium was maintained at 180 meq./day (Figure 1). During the two to four months which were spanned by these three periods, large variations of water intake were imposed and considerable losses of weight occurred; there were no significant changes in the average values of blood pressure or in the heart sizes (Table II).

When the sodium was reduced to the dietary level of about 7 meq./day by omission of the tablets, a statistically significant reduction of blood pressure was observed in each patient. Of the five patients three became normotensive (or nearly so), while the other two remained in the hypertensive range. The latter patients had the most marked elevations of blood pressure during the control periods and, therefore, would have had to experience greater reductions in order to approach the normal range.

Three of the four patients who were given salt tablets in the final period showed increases in their average blood pressures. Since the trend of the daily blood pressure values was upward in Period VI (Figure 3), it is probable that even higher averages would have been obtained in a more extended period. With the return of hypertension there were increases of the heart size which abolished earlier reductions (Table II).

*Weight losses* were observed in all patients (Figure 2). The graphical form of the weight curves was similar to that previously reported for a group of patients who received the rice-fruit diet (3); with both diets the weight losses proceeded at a rate which was rapid at first but which decreased with time. The rates of adaptation were so slow, however, that several additional months probably would have been required for attainment of definite weight equilibrium.

Since the caloric intakes of the patients remained under their control, it is evident that some factor in the regimen must have reduced their appetites. This factor certainly was not restriction of sodium since four of the five patients of the present study received 10 grams NaCl/day during the initial phase of rapid loss, and the remaining patient (C), who received no salt supplement, experienced a weight loss which was indistinguishable from the others. Moreover, the weight changes were not related to adaptation of the basal metabolic rates; these were measured at monthly intervals and were found to remain within normal limits (± 10 per cent) in all patients.

The explanation which seems most probable is that the limitation of protein intake caused the

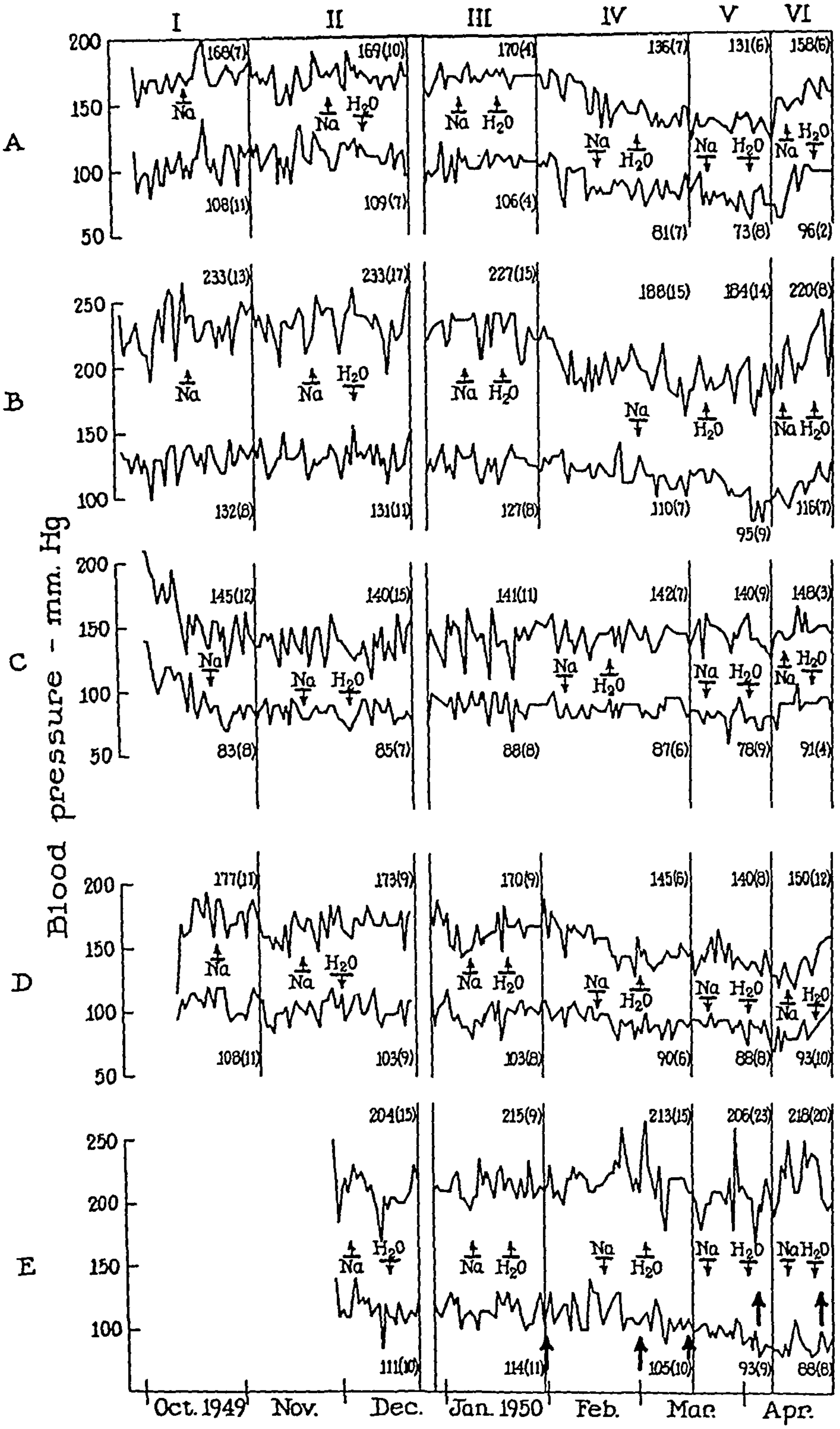

FIG. 1. MORNING VALUES OF THE SYSTOLIC AND DIASTOLIC BLOOD PRESSURES

The numbers near the end of each period are the averages and the standard deviations for the last two weeks of each period, except the last for which a one week average was taken. Sodium intake was 180 meq./day during periods with the arrow up, and 7 meq./day during those with arrow down. High and low water intakes, indicated similarly, were 4,500 cc./day and 800 to 1,000 cc./day, respectively.

weight losses. The basic diet was deficient in protein, as compared to the standard which was established by the patients themselves when given free choice in the matter, since each patient nearly doubled his protein intake when the protein supplement was made available for unlimited consumption (Figure 3, Periods III and IV). With the increased intakes of protein there were associated

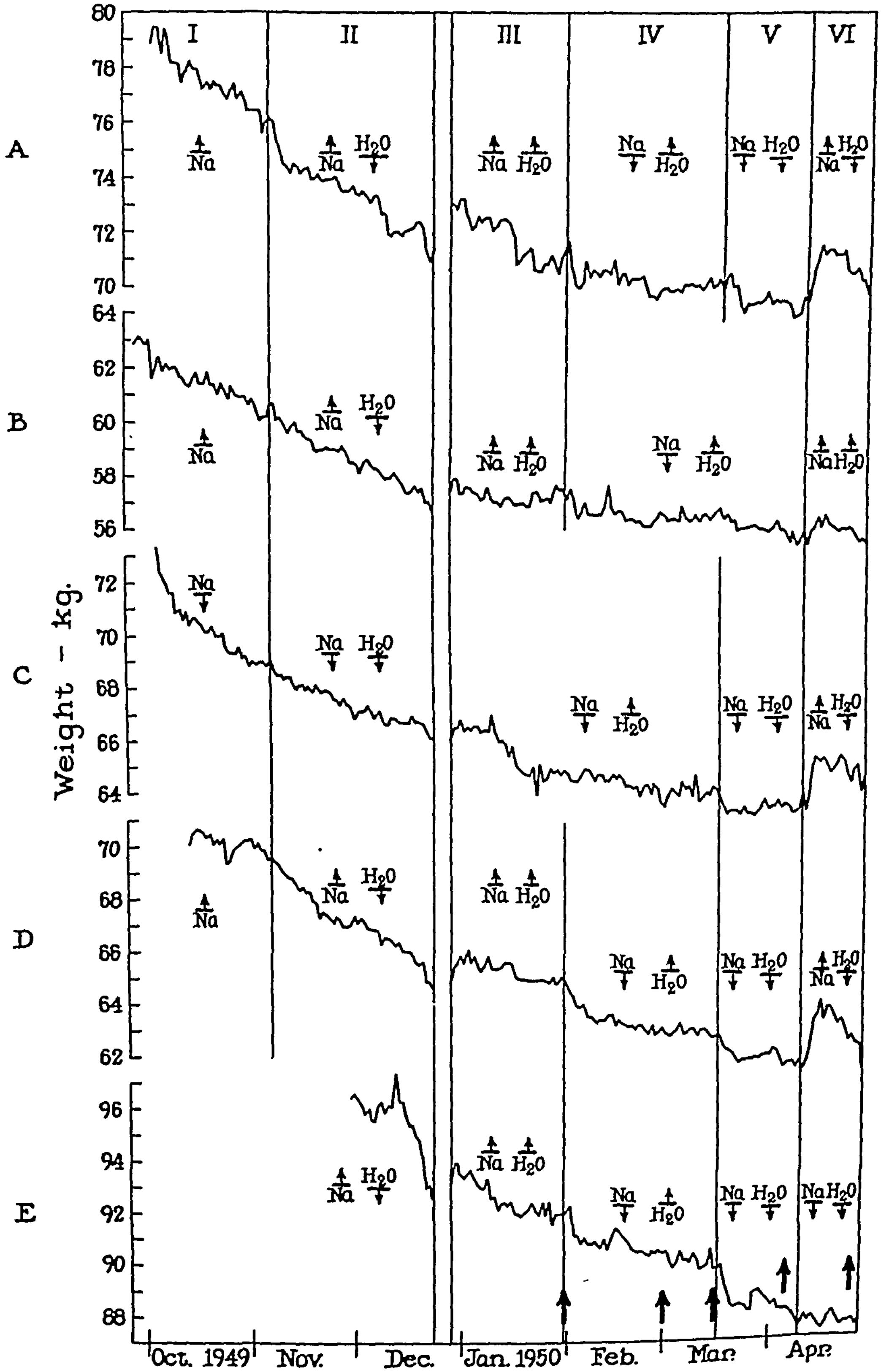

FIG. 2. MORNING WEIGHTS

Symbols are defined in the legend of Figure 1.

increases of non-protein calories, amounting to 100 to 300 calories per day.

A more direct demonstration of the relation between protein intake and change in body weight under the conditions of this study has been obtained in subsequent work. Three other patients, who were given the same diet under the same circumstances except that their protein intakes were maintained from the onset at 70–90 grams/day by a prescribed administration of the supplement, not only failed to lose weight but actually gained 1 to 4 kg. during a three month period of observation.

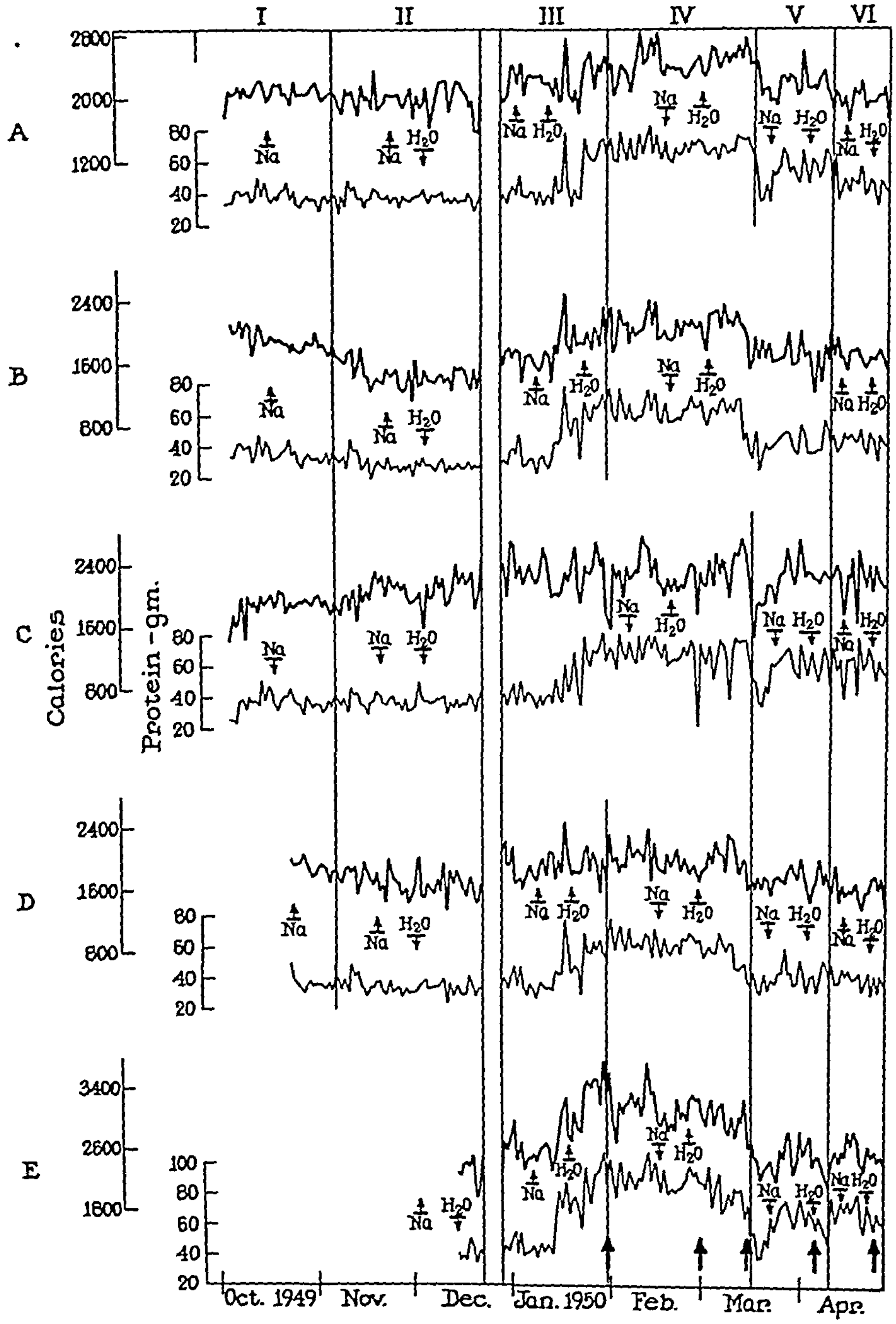

FIG. 3. CALCULATED DAILY CONSUMPTIONS OF TOTAL CALORIES AND OF PROTEIN
Symbols are defined in the legend of Figure 1.

*The changes in exchangeable sodium* are shown in Table II. The hypertensive patients, before omission of the salt supplement, did not differ from the three normal subjects with respect to the quantity of exchangeable sodium per unit body weight. These data do not exclude the possibility that a small difference between the average values of hypertensives and normals might be demonstrable in a larger series, but they indicate that the hypertensive subjects are not distinguished from the normals by a consistent or gross discrepancy.

The first measurements of exchangeable sodium were made at the end of Period III, two days before reduction of intake from 180 to 7 meq./day; the second were made one month later. In three of the four patients a reduction of 14–20 per cent was found. The explanation for the failure of Patient A to conform is not apparent; however, at the time of the third measurement two weeks later she was found to have a comparable reduction, while the other patients were unchanged. The fourth measurements were made three weeks later (a total of nine weeks from reduction of the sodium intake) and showed no further change in any of the patients.

The final measurements were made 16 days after the sodium intake had been returned to 180 meq./day. In the three patients (A, B, D) for whom the comparison could be made it was found that the exchangeable sodium had been returned to the control level.

The concentration of sodium in the serum was measured with each determination of exchangeable sodium. In contrast to the large changes which occurred in the latter, the concentration was not significantly affected by the variations of sodium intake.

*The urinary excretions of sodium* are shown graphically in Figure 4. Following reduction of their sodium intakes, the patients reduced their urinary excretions quite slowly: at least a week was required for reduction to the level of dietary intake. By the third week the urinary loss had fallen to 1 meq./day or lower, the output at this extremely low level being somewhat accelerated by a large urine volume and reduced with restriction of fluids.

After increase of the sodium intake in Period VI, the urinary excretions rose. Again there was a lag in the renal response to the changed situation. When the fecal excretions of sodium were measured during the last five days of Period IV and of Period VI, it was found that the daily losses by this avenue averaged less than 1.5 and 2.2 meq./day, respectively. Losses of this magnitude, although important with respect to a long-term balance under severe restriction of intake (3), evidently are negligible by comparison with the acute imbalances between intake and urinary excretion that were observed to follow a radical change in the level of intake. Therefore, since no gross sweating occurred during these observations, it is probable that the changes in the exchangeable sodium were due mostly to the lags in adjustment of the urinary excretion. Given time, the patients were able to resist further depletion, as shown by the constant values of the exchangeable sodium in the second, third, and fourth measurements; conversely, in the periods of high sodium intake they maintained a constant urinary excretion despite wide variations in their urine volumes.

It will be observed that the decreases in exchangeable sodium (Table II) were greater than the urinary excretions of the element during the first five days after reduction of intake (Table III). The probable reason for this is that the isotope equilibrations were attained in the afternoon, when the sodium ration of the day had been absorbed but not yet excreted (unpublished observations). The data for the excretions during the 14 hours that followed the last dose of NaCl were omitted from the calculations of Table III in order to present the ratios of sodium to chloride excretions on the subsequent days when no salt was administered.

*The urinary excretions of chloride* tended to parallel those of sodium. In particular it was noted that the net urinary losses of the two ele-

TABLE III

*Comparison of the net urinary losses of sodium and chloride during the five days following omission of salt tablets*

| Patient | Urinary output less dietary intake | | Na/Cl |
|---|---|---|---|
| | Na meq./5 days | Cl meq./5 days | |
| A | 131 | 82 | 1.60 |
| B | 144 | 119 | 1.21 |
| D | 146 | 132 | 1.11 |
| E | 166 | 115 | 1.44 |

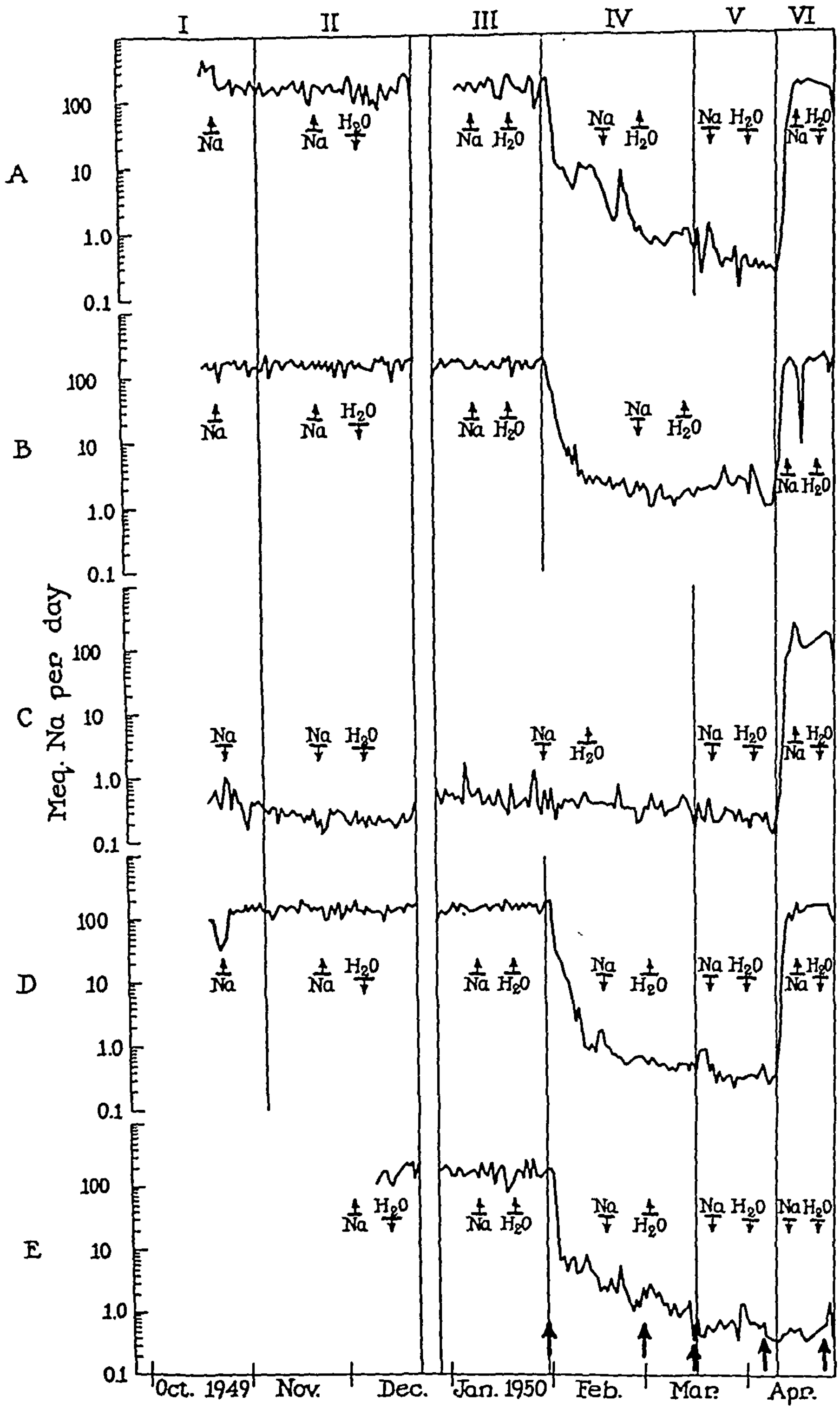

FIG. 4. URINE SODIUM EXCRETIONS

Note that the ordinate scales are logarithmic. Symbols are defined in the legend of Figure 1.

ments during the five days following reduction of intake occurred in ratios that approximated their ratios in extracellular fluid (Table III).

*The urinary excretions of potassium* tended to be greater during the early periods of rapid weight loss than subsequently. There was no clear relation of the urinary or fecal excretion of potassium to the changes in sodium intake.

## INTERPRETATION

The proportional relation between the sodium and the chloride excretions on the days that followed reduction in intake (Table III) suggests that the sodium, which was lost from the body early in Period IV, was derived from a source with the composition of extracellular fluid. A similar inference with respect to reaccumulation may be drawn from the immediate weight changes that follow readministration of salt. It was observed in an earlier study (3), and confirmed in the present one, that the patients for a time gain weight at a rate which is just sufficient to account for the formation of extracellular fluid from the newly-available sodium.

It will be assumed, therefore, that the component of the body which gained and lost sodium as a result of the dietary changes was the extracellular fluid. If 20 per cent of the sodium of the extracellular fluid were lost, it would cause a profound drop in the concentration of sodium in the serum (*e.g.*, to 115 meq./L or lower), unless the loss of sodium were compensated by a parallel loss of water. Therefore, since there were no significant changes in the concentration of sodium in the serum, it follows that water, as well as sodium, must have left the extracellular fluid at the time of the sodium loss, and conversely, that the extracellular volume must have expanded during reaccumulation.

It now becomes of interest to inquire into the fate of this extracellular water. If it had been excreted from the body, there would have been a weight loss of about 1 kg. for every 140 meq. of sodium which were lost, providing that an immediate large increase in the solid matter of the body had not occurred. The latter possibility seems quite unlikely, partly because the antecedent weight curves show that body tissue was being lost rather than gained at the time of the sodium depletion, and partly because this hypothesis leads to the unlikely supposition that this solid matter was abruptly expelled following the subsequent reaccumulation of sodium. Since the weight losses of all patients fell significantly short of equivalence with their losses of exchangeable sodium (Table II), the simplest hypothesis is that part or all of the water which left the extracellular space did not leave the body. Under this interpretation the average cellular hydration was increased by about 5 per cent as a result of the sodium depletion.

This hypothesis also simplifies the explanation of the biphasic nature of the weight change (Figure 2) following readministration of sodium. The initial effect, as mentioned above, probably is an expansion of the extracellular fluid. The secondary effect is a loss of the newly-gained weight but, as shown by the elevated final value of exchangeable sodium, not of the sodium which was accumulated. Therefore it appears that the water which entered the cells during the previous adaptation to a low sodium intake was removed from them and excreted during the secondary phase of readjustment to a high intake of sodium.

The general interpretation which may be given to these changes is that there is a reciprocal relation between the volume of the extracellular fluid and the volume of part or all of the cellular fluids. A radical reduction in the level of dietary sodium shifts the balance slightly in the direction of cellular hydration and a return to a high intake level reverses the shift.

## CLINICAL-METABOLIC CORRELATION

The persistent hypertension of all patients during the periods of high sodium intake was an impressive control for the reductions in blood pressure that occurred after omission of the salt tablets. Whether or not the antecedent two to four months of hospitalization, with the weight losses and the changes in protein metabolism, may have contributed to the ultimate therapeutic response cannot be said at present, but it is clear that these factors were not sufficient to reduce the blood pressure in the absence of sodium restriction.

The most obvious metabolic consequence of the reduction in sodium intake was the reduction in exchangeable sodium. However, this fact alone may not be a sufficient explanation of the clinical effect since there was no consistent correlation between the clinical status of the subjects and the quantity of exchangeable sodium per unit body weight. Moreover, the reductions in blood pressure and in exchangeable sodium were separated in time; the latter was mostly completed before the former became manifest.

Evidence has been brought forward to support the conclusion that the reduction of sodium intake caused an increase of intracellular water in addition to the contraction of extracellular volume. An indication that the change in intracellular water might be more closely related to the change in blood pressure than the change in extracellular volume is given by the delay in rise of blood pressure when salt was readministered; in this process it appears that the immediate effect is the expansion of extracellular volume and the delayed effect, which coincides with the blood pressure rise, is the expulsion of cellular water.

It is obvious that this hypothesis needs to be tested by direct measurements. Such test, however, must await the development of more precise methods for the measurement of small changes in the quantity of intracellular water in human subjects.

## SUMMARY

1. Restriction of dietary sodium to 7 meq./day caused statistically significant reductions in the average blood pressures of five patients with uncomplicated hypertension. These reductions were due to the limitation of dietary sodium, since no blood pressure changes had been observed during a preliminary equilibration stage of two to four months duration in which the low sodium diet was supplemented with salt tablets (10 grams/day). Weight losses and large variations in the intakes of protein and of water during this time were without significant effects on the blood pressure.

2. Following withdrawal of the salt supplement, the patients lost 14 to 20 per cent of their exchangeable sodium during the first month of adaptation and then apparently attained balance. On readministration of salt 10 weeks later, the patients reaccumulated sodium approximately to the control level.

3. On the supposition that the lost sodium was derived from extracellular fluid, the losses of body water appeared to be disproportionately small. The interpretation offered was that the surplus extracellular water, not excreted with its sodium, shifted into an intracellular position and thereby increased cellular hydration by about 5 per cent. The reverse effects were observed following readministration of salt. The hypothesis was advanced that the low sodium diet exerted its beneficial effect in the hypertensive patients by causing an increase in cellular hydration.

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# CONGENITAL SPCA DEFICIENCY: A HITHERTO UNRECOGNIZED COAGULATION DEFECT WITH HEMORRHAGE RECTIFIED BY SERUM AND SERUM FRACTIONS [1]

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(Submitted for publication January 17, 1951; accepted, March 26, 1951)

## INTRODUCTION

The importance of non-prothrombin plasma and serum constituents in the physiological conversion of prothrombin to thrombin has been recognized only recently. A hemorrhagic disorder, called "parahemophilia" (Owren's disease), is associated with an elevated prothrombin time attributable to deficiency of a plasma component termed "Factor V" (1, 2). Inadequacy of plasma Ac-globulin, required for rapid evolution of thrombin, has been described by Sykes and associates (3) in experimentally induced liver poisoning, and Alexander and Goldstein have reported on Labile Factor (Quick) deficiency in severe hepatic disease in man (4). Although conclusive evidence is lacking, most investigators agree that Factor V, Ac-globulin, and Labile Factor are identical. Subnormal concentrations result in retarded prothrombin conversion (elevated one-stage prothrombin time), in poor yield of thrombin by the two-stage prothrombin method, and in hemorrhagic phenomena despite the fact that the prothrombin level may actually be normal.

The clinical and laboratory investigation of a unique case herein reported provides substantial evidence that an additional constituent besides that (or those) described above is involved in prothrombin conversion, and that its lack may underlie a hemorrhagic diathesis, hitherto unrecognized, which simulates hypoprothrombinemia. Deficiency of Ac-globulin, Labile Factor, or Factor V were excluded as the basis for the clotting abnormality. The evidence warrants the conclusion that the defect consisted in derangement of the early activation of prothrombin due to congenital inadequacy of the precursor of the serum prothrombin conversion accelerator (spca) described in earlier reports (5–8).

## CASE REPORT [2]

R (No. 309157), a four year old white girl of Scotch, Irish, French and Dutch extraction, was admitted to the Children's Medical Center, Boston, on June 13, 1950, with bloody stools and hematemesis of three days' and six hours' duration, respectively. The family history was contributory only in the fact that the mother stated that she and her brother bruised easily and that the maternal grandfather died of intestinal bleeding following trauma.

The patient, an only child, was noted to have prolonged bleeding from the umbilical cord at birth. On the eighth and tenth days, she passed gross blood per rectum, which continued until the age of five weeks when she was first admitted to the Children's Medical Center. At that time her blood count, platelet count, and bleeding and clotting times were normal. The prothrombin was not determined. Although the barium enema was negative, an exploratory laparotomy was performed because of continued melena. No cause for the intestinal bleeding could be found, the abdominal organs appeared normal, and the appendix was removed. No undue hemorrhage was encountered, and convalescence was uneventful except for mild melena which continued for several months.

The patient's intervening history was perfectly normal save for easy bruising and occasional spontaneous epistaxis. Three weeks prior to her second admission she began to experience urinary urgency, frequency, and burning, unassociated with other symptoms. Five days prior to admission, she fell and sustained some bruises. The next day she passed four tarry stools, pallor was noted, and the patient became progressively listless. On the day of admission she began to bleed from the nose, vomited "coffee-ground" material on three occasions, and complained of crampy abdominal pain.

Physical examination revealed a fairly well developed, thin, very pale, quiet child who appearead acutely ill. T. 99° F.; pulse 130; respirations 30; B. P. 100/50. The skin showed a few small ecchymoses over the left leg, abdomen and forehead, but no petechiae. The nares con-

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[1] Aided by grants from the Commonwealth Fund and The United States Public Health Service.

[2] From the records of the Children's Medical Center.

tained a moderate amount of dry, clotted blood but no bleeding points were seen. Except for blood in the posterior nasopharynx, the throat was negative. The lungs were clear. The heart was slightly enlarged to the left, rate rapid, rhythm regular; a grade 2 systolic murmur was audible over the apex and left sternal border. The abdomen was completely negative; liver, kidneys and spleen were not palpable. There was no adenopathy. Rectal examination as well as the remainder of the physical examination was negative.

*Clinical laboratory data:* Rbc, 1.94; Hgb, 6.0 gm.; Wbc, 14,800 with normal differential; platelets appeared abundant on the stained smear; platelet count 412,000; reticulocytes, 7.5%; sedimentation rate, 4 mm. per hour; hematocrit, 17% packed cells. Six urines were completely negative; stools were repeatedly guaiac positive during the first three days but were negative thereafter. Hinton was negative. Liver function tests were within normal limits.

On admission, the bleeding time (Duke) was 12.5 minutes; the clotting time was six minutes; clot retraction was normal. The prothrombin time was 72 seconds (control 17).

X-ray examination of the abdomen, including barium enema, revealed normal findings.

*Clinical course:* A fresh whole blood transfusion of 500 ml. was slowly given, beginning at 2:15 P.M., June 13 and ending 1:20 A.M., June 14. The patient's clinical condition improved markedly. In addition, she received 4.8 mgm. Hykinone both subcutaneously and intravenously. At 7:00 A.M. on June 14 the prothrombin time was 55 seconds (control 16). The Rbc and Hgb had increased to 4.3 million and 14.8 gm., respectively. The bleeding time was six minutes.

On June 14, the patient was placed on Synkavite (menadione) 10 mgm. p.o.q. six hours and Hykinone 10 mgm. s.c.q. six hours. Another transfusion of 250 ml. of fresh whole blood was administered, beginning at 3:00 P.M. and ending 6:45 P.M. On June 16 the prothrombin time was 64 seconds (control 15).

After considerable improvement, with no recurrence of hematemesis or melena, the patient was discharged on the eighth day. She was seen[3] again four months later at which time her clotting defect was found relatively unchanged; her prothrombin time was 62 seconds, her coagulation time (Lee-White, 37° C), 17 minutes (normal: 4–12 minutes.). The investigation of the coagulation defect is recorded below.

## METHOD

Prothrombin was determined by both the one- and two-stage methods. In the former, two procedures were employed: the prothrombin time was observed on whole oxalated plasma (1 part 0.1 M sodium oxalate to 9 parts venous blood), and on whole plasma diluted with pooled oxalated normal plasma rendered prothrombin free by adsorption with $BaSO_4$ (hereafter referred to as $BaSO_4$ plasma), according to techniques previously described (9). In some experiments, the patient's plasma was mixed with normal plasma unadsorbed with $BaSO_4$. Commercial rabbit brain thromboplastin (Difco) was used throughout except where indicated otherwise.

In the two-stage procedure, also, two methods were used: the orthodox technique (10), and the modification of Ware and Seegers (11) in which optimal amounts of Ac-globulin are provided by bovine $BaCO_3$ adsorbed serum (1 to 150 dilution). Discrepancies between the results reflect inadequacy of Ac-globulin in the system not containing the bovine serum. Beef lung provided the source of thromboplastin, and Armour Fraction I provided the fibrinogen.

Plasma Labile Factor (L.F.) was measured by the ability of the plasma to rectify the retarded prothrombin conversion of aged normal oxalated pooled plasma in which this clotting component had deteriorated (12, 13).

The serum prothrombin conversion accelerator (spca) was determined by a method previously described (5). Serum devoid of spca was prepared by adsorption with $BaSO_4$ (100 mgm. per ml.) for ten minutes at room temperature.

Preparations of prothrombin were obtained from plasma by adsorption with $BaSO_4$ and elution with 5% solutions of sodium citrate in physiological saline, as described in earlier reports (14, 8). Purified spca was similarly prepared from serum according to published methods (8).

## RESULTS

*One- and two-stage plasma prothrombin values:* The prothrombin time of the patient's whole plasma was repeatedly found elevated to approximately 70 seconds consistent with a prothrombic activity of about 3% of normal (Table I). This was not referable to prothrombin deficiency, how-

TABLE I

*Patient's plasma prothrombic activity*

| Date of determination | Prothrombic activity | | | |
|---|---|---|---|---|
| | One-stage | | Two-stage | |
| | Proth. time | Proth. | Without Ac-glob.† supplement | With Ac-glob.† supplement |
| | *sec.* | *per cent* | *units per ml.* | *units per ml.* |
| 6/20/50 | 72 | 3 | 170 | 214 |
| 6/26/50 | 69 | 3 | 170 | 206 |
| 9/29/50* | 77 | 2 | | |
| 6/27/50 | 75 | 2 | 184 | 200 |
| 11/2/50 | 62 | 4 | 200 | 225 |

* Plasma of 6/26/50 kept in frozen state at −10° C, thawed and tested on 9/29/50.

† Ac-globulin supplement consists of $BaCO_3$ bovine serum.

In our laboratory the prothrombic activity of normal plasma is: one-stage, 80–120%; two-stage, 160–220 units per ml.

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[3] At the Yamins Research Laboratory.

TABLE II

*Prothrombic activity of patient's plasma in combination with normal plasma*

| Plasma mixture (parts) | | | | Proth. time* (*sec.*) |
|---|---|---|---|---|
| Norm. plas. | Norm. $BaSO_4$ plas. | Pt.'s plas. | Pt.'s $BaSO_4$ plas. | |
| 1 | 9 | — | — | 34 |
| 1 | — | — | 9 | 32 |
| — | 9 | 1 | — | 120+ |
| — | 2 | 1 | — | 100 |
| 1 | 9 | — | — | 34 |
| 1 | — | — | 9 | 38 |
| 1 | — | 1 | — | 19 |
| 1 | — | 4 | — | 23 |
| 1 | — | 9 | — | 26 |
| 1 | 9 | — | — | 31 |
| 1 | 4 | — | — | 23 |
| 1 | — | — | — | 17 |
| — | — | 1 | — | 72 |

* Prothrombin time observed on 0.1 ml. plasma mixture added to 0.1 ml. thromboplastin, followed by the addition of 0.1 ml. of 0.025 M $CaCl_2$. This obtains also for Tables *III, IV, VI, VII, VIII.*

ever, since by the two-stage method the prothrombin level was consistently within normal limits.

### *Exclusion of disturbance in thrombin-fibrinogen interaction, antithromboplastin, or anticoagulant*

The elevated prothrombin time could be due either to retarded evolution of thrombin or to some disturbance in the thrombin-fibrinogen interaction. The latter was readily excluded. First, minute amounts of thrombin (Parke-Davis Topical Thrombin) clotted the patient's plasma as rapidly as normal plasma. Second, the prothrombin times of mixtures of normal plasma with the *patient's* plasma, whole or deprothrombinated by $BaSO_4$ were no greater than those obtained on similar mixtures of normal plasma with *normal* $BaSO_4$ plasma (Table II). Thus, the clotting defect could not be related to abnormalities in the patient's fibrinogen or antithrombin, to antithromboplastin, or, for that matter, to the presence of any other anticoagulant.

It was obviously necessary to exclude the possibility that the patient's prothrombin might have been resistant to rabbit brain thromboplastin (used in the one-stage method) in contrast to bovine lung thromboplastin (in the two-stage procedure). This hypothesis became untenable when it was found that the prothrombin time was still far above normal when bovine lung thromboplastin was substituted for rabbit brain (Table III). Similar results were also obtained with human brain thromboplastin (acetone dehydrated). Curiously, whereas human thromboplastin was more effective than bovine thromboplastin on *normal* plasma, it was less effective on the patient's plasma. The explanation for this is obscure.

*Exclusion of Ac-globulin deficiency:* The question arose as to whether the retarded thrombin evolution was due to inadequacy of any of the known prothrombin-conversion factors. Deficiency of Ac-globulin was easily excluded: two-stage plasma prothrombin determinations by the orthodox procedure, in which supplements of Ac-globulin are not provided, gave values almost as good as those obtained with added Ac-globulin (Table I, Figure 1). The slightly lower values are not unusual in our experience with hundreds of measurements on normal plasma, and are in accordance with the finding of Murphy and Seegers that normally the concentration of Ac-globulin in man is critically low (15). Also noteworthy is the normal activation rate of the prothrombin within the interval observed (Figure 1). This was found repeatedly.

*Exclusion of labile factor deficiency:* L. F. concentration was also relatively normal. The restorative activity of the patient's plasma, whole or treated with $BaSO_4$, on the retarded prothrombin conversion of aged plasma, was approximately 70% of normal (Figure 2), a level not infrequently observed in patients with active or recent bleeding. This concentration is certainly sufficient to assure a normal prothrombin time if other clotting constituents are normal (16, 12). That the coagu-

TABLE III

*Prothrombic activity of patient's plasma with thromboplastin (Tpl.) from different species*

| | Prothrombin time (*sec.*) | | |
|---|---|---|---|
| | Human brain Tpl. | Rabbit brain Tpl. | Bovine lung Tpl. |
| Norm. plas. | 17 | 19 | 25 |
| Norm. plas. (*1*) plus norm. $BaSO_4$ plas. (*9*) | 33 | 29 | 53 |
| Pt.'s plas. | 155 | 77 | 56 |
| Pt.'s plas. (*1*) plus norm. $BaSO_4$ plas. (*2*) | 175 | 77 | 62 |

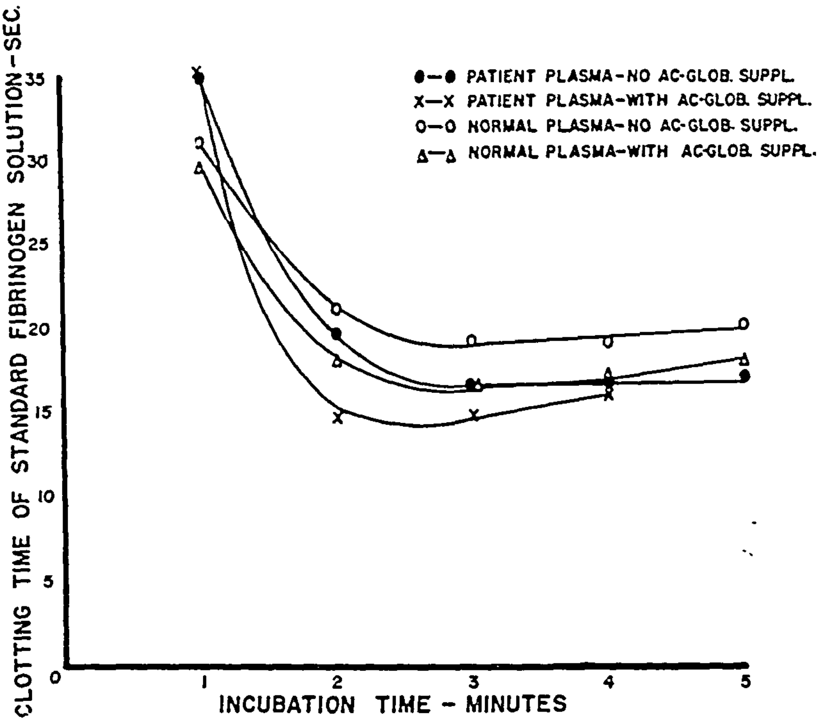

FIG. 1. TWO-STAGE PROTHROMBIN CONVERSION OF PATHOLOGICAL COMPARED WITH NORMAL PLASMA

Ac-globulin supplement consists of bovine $BaCO_3$ adsorbed serum (11). Prothrombin activation performed on a 1 to 40 dilution of test plasma with saline or with a 1 to 150 bovine serum-saline mixture.

lation defect could not be attributed to L. F. deficiency is established by the observation that diluting the patient's plasma with $BaSO_4$ normal plasma, which contains a full complement of L. F. (16, 17) failed to rectify the abnormality (Table II). Also, diluting normal plasma with the *patient's* $BaSO_4$ plasma gave essentially the same prothrombin times as were obtained on normal plasma similarly diluted with *normal* $BaSO_4$ plasma (Table II). It is, therefore, concluded that the clotting defect was not related to deficiency of Ac-globulin, of L. F., or of any entity contained in $BaSO_4$ adsorbed normal plasma.

*Exclusion of Factor V deficiency:* Although it is likely that Ac-globulin and L. F. are identical with Factor V, inadequacy of the latter had to be ruled out by methods employed by Owren in his case of parahemophilia (1, 2). In his subject, the elevated prothrombin time could be rectified by admixture of deprothrombinated Seitz-filtered normal plasma with the pathological plasma. Accordingly, 35 ml. of normal oxalated plasma were passed through a Seitz filter. The first 5 ml. were discarded, and the remainder was refiltered through the same pad. The filtrate was prothrombin-free but contained L. F. and Ac-globulin activity. A mixture of 1 part of pathological plasma with 2 parts of normal Seitz-filtered plasma gave a prothrombin time of 88 seconds; a mixture of 1 and

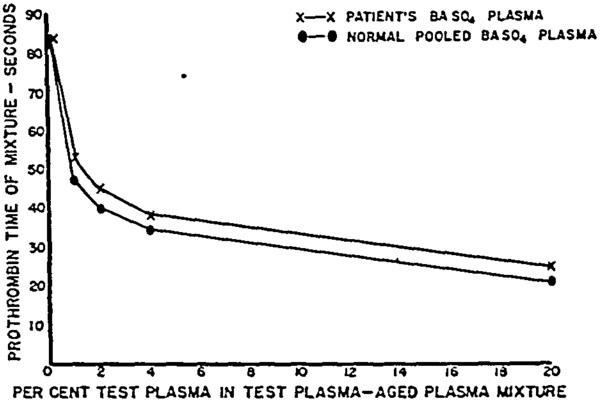

FIG. 2. LABILE FACTOR OF PATHOLOGICAL COMPARED WITH NORMAL PLASMA

Test material added in various concentrations to normal pooled oxalated plasma aged for five weeks at 4-5° C.

TABLE IV

*Activity of prothrombin fraction separated from patient's plasma*

Prothrombin fraction dissolved in 0.14 M sodium citrate and 0.15 M NaCl equivalent in volume to the plasma from which it was derived. The solution contained 184 units of prothrombin per ml. compared with 214 units per ml. in the parent plasma.

| Mixture (parts) | | | | Proth. time (*sec.*) |
|---|---|---|---|---|
| Pt.'s plas. | Pt.'s proth. | Norm. plas. | $BaSO_4$ norm. plas. | |
| 1 | — | — | — | 72 |
| 1 | — | — | 9 | 93 |
| — | 1 | — | 4 | 85* |
| — | 1 | — | 18 | 98 |
| — | — | 1 | 19 | 47 |
| 1 | — | 1 | 18 | 33 |
| — | 1 | 1 | 18 | 34 |

* Subsequent determinations gave progressively increasing prothrombin times, indicating rapid deterioration of the prothrombic activity. This phenomenon was observed repeatedly.

9, respectively, failed to clot in six minutes. On the basis of these and other observations (see below), parahemophilia was excluded.

### *Possibility of qualitative defect in patient's prothrombin*

Conceivably the patient's prothrombin might have been inherently defective, and thus in its early activation only slowly susceptible to the action of thromboplastin and calcium. This might escape detection in the two-stage method where, although thrombin evolution takes place, it is not discernible until after a longer interval (minutes) of interaction between the coagulation components than in the one-stage system (seconds). To test this possibility, the patient's prothrombin was separated and purified. The prothrombin, which was thus obtained in full yield, behaved like that in the whole plasma: it was normal when measured in the two-stage system but when it was added to $BaSO_4$ normal plasma its one-stage conversion was still retarded (Table IV). The possibility still remained, therefore, that the prothrombin molecule itself was abnormal. Clearly, this question can be conclusively answered only by testing the prothrombin in an ideal diluent consisting of hypoprothrombinemic plasma assuredly containing *all* the accessory prothrombin factors.

*Patient's prothrombin consumption:* Although two-stage prothrombin conversion was normal, it was of interest to see whether the retarded one-stage thrombin formation was associated with decreased prothrombin consumption during the course of *spontaneous* coagulation. Accordingly, the following experiments were performed: venous blood, drawn with a siliconized syringe and needle, was allowed to clot in clean non-siliconized dry test tubes. At specific intervals after the blood was withdrawn, oxalate was added, the mixture was stirred with a glass rod, and the tubes were centrifuged. Residual prothrombin in the sera was determined by the modified two-stage method. Contrary to what was expected, prothrombin consumption was found perfectly normal (Figure 3). This indicates that if prothrombin conversion was retarded, it was so only in an early phase of the clotting process, perhaps within that interval represented by the one-stage prothrombin time where *initial* thrombin formation is detected. Apparently, as "clotting" proceeded, the velocity of thrombin elaboration increased and soon became normal. This interpretation is consistent with the normal plasma prothrombin values obtained by the two-stage method, where normal activation and maximal yield of thrombin was evident within a few minutes after thromboplastin and calcium were added to the system (Figure 1). Under the conditions of the two-stage procedure, a disturbance in the earliest phase of prothrombin activation could escape detection.

*Spca mechanism in the pathological blood:* It has previously been shown that spca evolves in re-

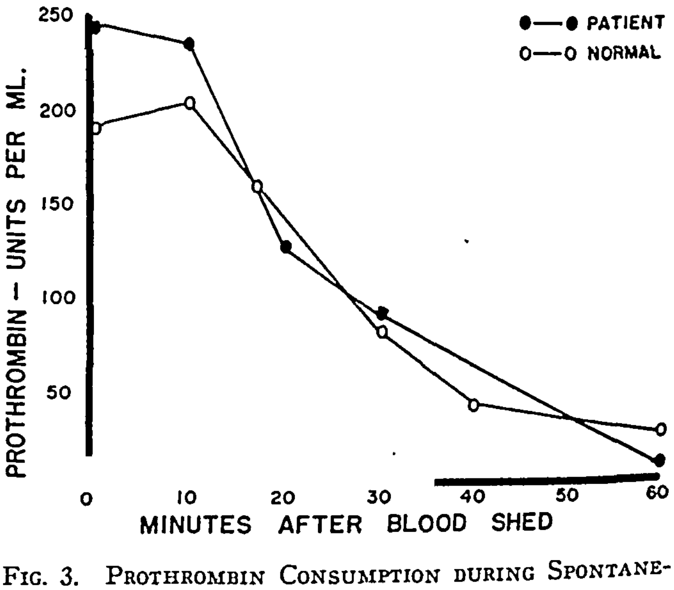

FIG. 3. PROTHROMBIN CONSUMPTION DURING SPONTANEOUS COAGULATION

TABLE V

*Spca in patient's serum*

| Date | Spca (*Units per ml. of serum*)* | | | |
|---|---|---|---|---|
| | 10′ | 20′ | 30′ | 60′ |
| 6/20 | — | — | 15 | 0 (0)† |
| 6/26 | — | — | — | 2 |
| 6/27 | — | — | 2 | 2 |
| 11/2 | 11 | 7 | 0 | 8 |

Serum oxalated and separated at indicated interval after blood was shed.

* Normal serum contains 14–40 units of spca per ml.

† Value in parentheses obtained on serum from an aliquot of blood (4.5 ml.) placed in tube containing thromboplastin (0.5 ml.) prepared as for prothrombin determination. The effect on the clotting time was not observed in this instance, but on another occasion 0.2 ml. of thromboplastin added to 2.0 ml. of the patient's blood lowered the clotting time from 17 to 2½ minutes. Under these conditions normal blood would have clotted almost instantaneously.

lation to the amount or velocity of prothrombin conversion (6, 7). This substance, distinct from Ac-globulin, is considered by us to be the "autocatalytic" accelerator of thrombin formation (8). Arising from an as yet unidentified precursor in plasma, it apparently potentiates the action of thromboplastin (8). Both spca and its progenitor are adsorbable by $BaSO_4$ (5, 7, 8, 14).

In view of the role of spca in prothrombin conversion, it was of interest to study the elaboration of spca during coagulation of the patient's blood. The spca activity of her serum was generally abnormally low despite normal prothrombin consumption (Table V). It was also noteworthy that accelerating the clotting of her freshly shed blood with thromboplastin supplements failed to increase the spca, in contrast to what is obtained on normal blood (6). However, on one occasion, 30 minutes after the blood had been shed, spca activity appeared normal, but within the next 30 minutes it had decreased markedly. It was, therefore, possible that the patient's plasma may have exerted a destructive action on whatever spca was elaborated. This was excluded by experiments in which purified spca, separated from normal serum, was incubated at 37° C with the pathological plasma. No deterioration occurred in three hours.

*Effect of storage on patient's plasma:* Plasma

TABLE VI

*Effect of normal serum and purified spca on patient's prothrombic activity (one stage)*

| Mixture (parts) | | | | | | | | | Proth. time (*sec.*) |
|---|---|---|---|---|---|---|---|---|---|
| Pt.'s plas. | Pt.'s ser.* | Norm. plas. | Norm. ser.† | Norm. $BaSO_4$ ser. | Pt.'s proth. prep.‡ | Spca prep.§ | Saline | Norm. $BaSO_4$ plas. | |
| 1 | — | — | — | — | — | — | — | — | 72 |
| 1 | — | — | — | — | — | — | — | 9 | 93 |
| 1 | — | 1 | — | — | — | — | — | — | 19 |
| 1 | — | — | 1 | — | — | — | — | — | 15 |
| 1 | 1 | — | — | — | — | — | — | — | 63 |
| — | — | 1 | 1 | — | — | — | — | — | 13 |
| 1 | — | — | — | — | — | — | 1 | 18 | 180+ |
| 1 | — | — | 1 | — | — | — | — | 18 | 28 |
| — | — | 1 | — | — | — | — | 1 | 18 | 47 |
| — | — | 1 | 1 | — | — | — | — | 18 | 23 |
| 1 | 1 | — | — | — | — | — | — | 18 | 95–130 |
| — | 1 | 1 | — | — | — | — | — | 18 | 44 |
| 1 | — | — | — | — | — | 1 | — | 18 | 31 |
| — | — | 1 | — | — | — | 1 | — | 18 | 28 |
| — | — | — | — | — | 1 | — | — | 4 | 85 |
| — | — | — | 1 | — | 1 | — | — | 18 | 32 |
| — | — | — | — | 1 | 1 | — | — | 18 | 86–130 |
| — | — | — | — | — | 1 | 1 | — | 18 | 24 |
| 1 | — | — | — | — | — | — | — | 1 | 93 |
| 1 | — | — | — | — | — | 1 | — | — | 14 |

* Obtained 60 minutes after blood withdrawn. Contained 2 units prothrombin per ml. (see data of 6/20—Table V).

† Obtained 60 minutes after blood withdrawn. Contained 6 units prothrombin per ml.

‡ Same preparation as described in Table IV. Obtained from patient's plasma used in this experiment.

§ Solution containing 1 mgm. protein, 38 units spca, per ml.

prothrombic activity, as measured by the orthodox one-stage method, decreases during storage because of deterioration of L. F. (12, 14, 18). When, however, determinations are made by the modified procedure in which the aged plasma is diluted with fresh $BaSO_4$ plasma, the stored plasma shows *increased* prothrombic activity. Such "hyperactivity" also develops in aging prothrombin fractions obtained by $BaSO_4$ adsorption, and elution with citrate (14). This is attributable to evolution of a prothrombin conversion accelerator, which because of its resemblance to spca with regard to activity, stability and adsorbability by $BaSO_4$, has been identified with spca.

This phenomenon was not demonstrable in the pathological plasma: during storage of the oxalated plasma in glass at refrigerator temperature for 25 days, no significant change in prothrombic activity was observed.

*Effect of spca on the clotting defect:* All of these findings suggested a derangement in the spca mechanism. Experiments were therefore performed to ascertain whether normal serum rich in spca could affect prothrombin conversion in the patient's plasma. When normal serum was mixed with the pathological plasma, the prothrombin time was markedly decreased (Table VI). In contrast, the patient's serum was relatively inert on both her own as well as on normal plasma. Normal serum also induced more rapid thrombin evolution from a prothrombin preparation derived from the abnormal plasma, and the same results were obtained when purified spca fractions were substituted for normal serum. Furthermore, removal of spca from normal serum by $BaSO_4$ adsorption rendered it inert. The clot-promoting effect can therefore be attributed to spca rather than to another serum component.

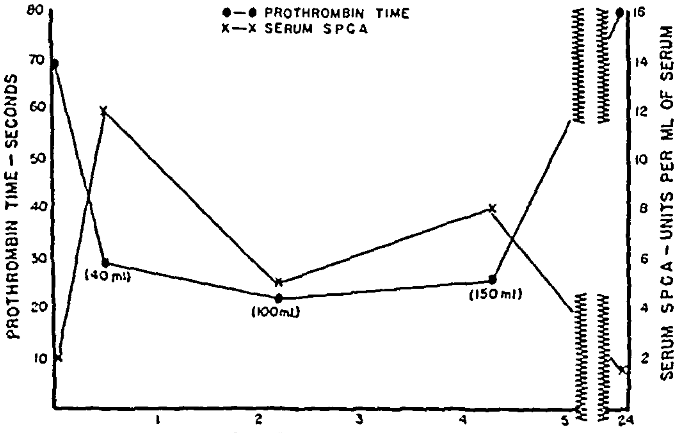

FIG. 4. EFFECT OF SERUM TRANSFUSION ON PATIENT'S PROTHROMBIN TIME AND SPCA

*Effect of serum in vivo:* Whether or not a defective spca mechanism was the fundamental disturbance, the *in vitro* effect of normal serum suggested its use as a therapeutic agent. Accordingly, five days after discharge the patient was readmitted to the Children's Medical Center to receive a slow infusion of 150 ml. of 24 hour old normal serum. The prothrombin time decreased markedly, and remained lowered for several hours, but gradually increased to its previous level within 24 hours (Figure 4). Concomitant with the rise in one-stage prothrombic activity, the patient's serum spca increased, only to drop rapidly as the beneficial effect of the serum wore off. The two-stage prothrombin values were unchanged.

*Clot-promoting effect of plasma compared with serum:* It is not clear whether a transfusion of normal blood or plasma would have had the same effect. From the clinical record it appears that even a larger amount of plasma (approximately 250 ml. from 500 ml. of blood) was ineffective although the interval between the end of the transfusion and the subsequent prothrombin determination (approximately six hours) was too long to warrant a definitive conclusion. However, that no significant improvement in clotting was evident six hours after the transfusion serves to differentiate this case from Owren's subject in whom the clot-promoting effect was still detectable several days after a blood transfusion.

Clearly, the administration of normal plasma should have some beneficial effect since mixtures of normal with the pathological plasma exhibit at least the full prothrombic activity of the normal plasma (Table II). The question arises whether *more* activity is demonstrable than can be accounted for by the normal plasma alone, *i.e.*, whether normal plasma *accelerates thrombin evolution from the patient's prothrombin.* It would appear that normal plasma was relatively inert in this regard (Table II). This, also, is in sharp contrast to what Owren observed in parahemophilia (1). Some observations, however, indicate that thrombin formation was accelerated under certain circumstances. A 1 to 20 dilution of normal plasma with $BaSO_4$ normal plasma gave a prothrombin time of 47 seconds (Table IV). When one part of the pathological plasma was incorporated into the mixture, the prothrombin time was 33 seconds. This difference, constituting a considerable increase in prothrombic activity, was found also with the patient's prothrombin fraction.

The question whether normal plasma contains an entity deficient in the patient's plasma and capable of correcting its disturbed clotting, seemed sufficiently important to warrant more detailed study. Accordingly, normal plasma was mixed in various dilutions with the pathological plasma, and the prothrombin times of the mixtures were compared with those of normal plasma similarly diluted with prothrombin-free $BaSO_4$ normal plasma (Table VII). In mixtures containing less than 10% normal plasma, shorter prothrombin times were observed on normal-pathological plasma mixtures than on the normal-$BaSO_4$ normal plasma counterparts (31 seconds *vs.* 39; 34 *vs.* 54; 36 *vs.* 90). It should be emphasized that these differences, repeatedly observed, are definitely outside the experimental error of the method.

TABLE VII

*Thrombin evolution in various combinations of pathological plasma with normal plasma or serum*

| Plasma mixture (parts) | | | | | Proth. time (*sec.*) |
|---|---|---|---|---|---|
| Norm. plas. | Pt.'s plas. | Norm. ser.* | Norm. $BaSO_4$ plas. | Norm partial† $BaSO_4$ plas. | |
| 1 | — | — | — | — | 17 |
| — | 1 | — | — | — | 74 |
| — | — | — | 1 | — | >360 |
| — | — | — | — | 1 | 70 |
| 1 | — | — | 1 | — | 17 |
| 1 | — | — | — | 1 | 16 |
| 1 | 1 | — | — | — | 17 |
| — | 1 | — | 1 | — | 93 |
| 1 | — | — | 4 | — | 22 |
| 1 | — | — | — | 4 | 20 |
| 1 | 4 | — | — | — | 21 |
| — | 4 | 1 | — | — | 17 |
| 1 | — | — | 9 | — | 28 |
| 1 | — | — | — | 9 | 27 |
| 1 | 9 | — | — | — | 25 |
| — | 9 | 1 | — | — | 20 |
| 1 | — | — | 19 | — | 39 |
| 1 | — | — | — | 19 | 33 |
| 1 | 19 | — | — | — | 31 |
| — | 19 | 1 | — | — | 25 |
| 1 | — | — | 29 | — | 54 |
| 1 | — | — | — | 29 | 40 |
| 1 | 29 | — | — | — | 34 |
| — | 29 | 1 | — | — | 30 |
| 1 | — | — | 39 | — | 90 |
| 1 | — | — | — | 39 | 43 |
| 1 | 39 | — | — | — | 36 |
| — | 39 | 1 | — | — | 30 |
| — | 1 | — | 9 | — | 144 |
| — | 1 | — | 19 | — | 270 |

* Normal non-oxalated serum, devoid of prothrombin, 24 hours old.

† Normal plasma adsorbed with 15 mgm. $BaSO_4$ per ml. for approximately 10 minutes at 4–5° C.

It was conceivable that the faster thrombin evolution in the normal-pathological mixtures may have been due to the additive effect of the small yet significant prothrombic activity of the abnormal plasma with its prothrombin time of 74 seconds in contrast to that of the $BaSO_4$ normal plasma (> 360 seconds). Attempts were therefore made to obtain (for the purpose of a diluent) a $BaSO_4$ normal plasma with a prothrombin time more comparable to that of the pathological plasma. After considerable trial and error, this was accomplished by adsorbing normal plasma with 15 mgm. $BaSO_4$ (C. P.-Baker) at 4–5° C for approximately ten minutes. The adsorbed plasma had a prothrombin time of 70 seconds. The prothrombin times of normal-pathological plasma mixtures were also shorter than those of normal plasma similarly diluted with this hypoprothrombinemic plasma (Table VII). Here, too, the differences were evident only at high dilutions, *i.e.*, in mixtures with prothrombin times exceeding 30 seconds.

Additional experiments were performed: 1 part of normal plasma was mixed with 1 part of pathological plasma. The ensuing mixture was then diluted with various amounts of prothrombin-free $BaSO_4$ normal plasma, and the prothrombin times were compared with those simultaneously obtained on normal plasma diluted with the $BaSO_4$ normal plasma. In this way, the same diluent was provided to both the normal plasma and the normal-pathological combination. Again, prothrombin conversion seemed faster in mixtures containing both normal and pathological plasma (Figure 5), particularly in the range where the prothrombin time exceeded 28 seconds. Mixtures of pathological plasma alone with the $BaSO_4$ normal plasma gave markedly elevated prothrombin times, which became progressively greater as dilution with the $BaSO_4$ normal plasma was increased.

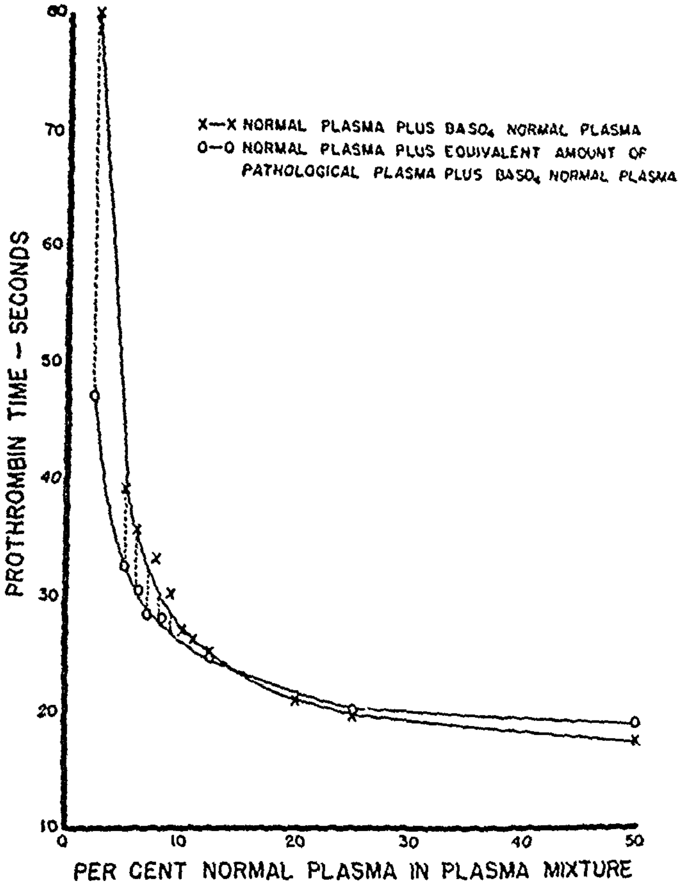

FIG. 5. PROTHROMBIN TIMES OF MIXTURES OF PATIENT'S PLASMA, NORMAL PLASMA, AND $BaSO_4$ NORMAL PLASMA

Curve with crosses represents normal plasma diluted in $BaSO_4$ normal plasma. Ten% normal plasma in this mixture signifies 1 part normal plus 9 parts $BaSO_4$ normal; 20%, 1 normal plus 4 $BaSO_4$ normal; . . . etc.

Curve with circles represents observations on normal plus pathological plasma, diluted in $BaSO_4$ normal plasma. Ten% normal plasma in this mixture signifies 1 part normal, 1 part pathological and 8 parts $BaSO_4$ normal plasma; 20% represents 1 part normal, 1 part pathological and 3 parts $BaSO_4$ normal, . . . etc. Fifty% represents 1 normal plasma plus 1 pathological plasma.

It might still be argued, nevertheless, that the conditions of the foregoing experiments were not strictly comparable since the pathological plasma contained normal amounts of prothrombin whereas the $BaSO_4$ plasma contained little or none. Obviously, this objection could be met only by having available pure prothrombin which could be incorporated into the various plasma mixtures in order to attain *comparable* prothrombin concentrations.

There can be little doubt, however, that to a certain extent normal plasma can truly rectify the retarded prothrombin conversion of the abnormal plasma. It is, however, far less effective than serum (Table VII). Furthermore, the clot-promoting effect of serum, and also of spca preparations, was observable in all ranges of prothrombin time, whereas the nomal plasma seemed effective only in mixtures with prothrombin times in excess of approximately 30 seconds. The explanation of this is obscure. Either the correcting factor in normal plasma is present in critical amounts, insufficient to handle substantial amounts of the additional prothrombin provided by the patient's plasma, or a certain interval (about 30 seconds) is required after the addition of thromboplastin and calcium for the corrective effect of normal plasma to supervene. Certainly the prothrombin method is sufficiently sensitive to have permitted detection of clot-promoting effect also in ranges where the prothrombin times of mixtures were between 20 and 30 seconds. Thus, in the presence of an amount of normal plasma which would give a prothrombin time of *less* than 30 seconds, the corrective effect of the factor in normal plasma may not be evident if 30 seconds or more is required for its conversion to the component active in correction of the clotting defect.

Regardless of whether this interpretation is correct, the clot-promoting effect of normal plasma provides strong evidence against the hypothesis that the patient's prothrombin was inherently defective. It is more reasonable to conclude that the abnormality lay in deficiency of a coagulation factor present in normal plasma, but absent in $BaSO_4$ plasma.

*Clot-promoting effect of dicumarol plasma:* Consistent with this conclusion is the observation that hypoprothrombinemic dicumarol plasma also

TABLE VIII

*Prothrombic activity of patient's plasma mixed with dicumarol plasma*

| Plasma mixture (parts) | | Dicum. $BaSO_4$ plas. | Proth. time (*sec.*) |
|---|---|---|---|
| Pt.'s plas. | Dicum.* plas. | | |
| 1 | — | — | 72 |
| — | 1 | — | 37 |
| 1 | 1 | — | 27 |
| 1 | — | 1 | 41 |

* Plasma from subject with myocardial infarct under treatment with dicumarol for prevention of thromboembolic disease. Plasma prothrombic activity—8% of normal (one-stage).

could induce thrombin evolution in the patient's plasma (Table VIII). This finding, furthermore, reflects a striking difference between dicumarol plasma and normal plasma deprothrombinated by $BaSO_4$. Moreover, $BaSO_4$ adsorption of the dicumarol plasma decreases its effect. This lends further support to the concept that the clotting factor present in normal plasma and deficient in the patient is adsorbable by $BaSO_4$.

## DISCUSSION

Recent recognition of the importance of nonprothrombin factors in the conversion of prothrombin has permitted more precise delineation of the coagulation defect in certain patients who formerly would have been erroneously grouped in one category, namely, hypoprothrombinemia. Exhaustive study of these rare cases has led, at least in one instance (1), to the discovery of hitherto unknown clotting components.

The observations contained in this report indicate the existence of another prothrombin-conversion factor besides those already reported. Deficiency in this entity (or its deranged function) results in early retardation of thrombin evolution, a defect which is associated with hemorrhagic phenomena despite essentially normal concentrations of prothrombin, Ac-globulin, L. F., and Factor V.

The existence of another prothrombin-conversion plasma component was postulated by Quick on the basis of observations on two brothers with abnormal bleeding and elevated prothrombin times (19). This moiety, originally termed "prothrombin A," is not reduced by dicumarol in contrast to "prothrombin B," which is probably identical with the purified prothrombin of Seegers, Loomis and Vandenbelt (20). Prothrombin A is said to be adsorbed by alumina and calcium phosphate together with Prothrombin B, in contradistinction to L. F. (19). The subject has been further complicated by the most recent change in terminology by Quick and Stefanini who now consider the total "prothrombin complex" to comprise three components: prothrombin "A," prothrombin "pre-A" and L. F. (18).

Alexander and associates, also, have presented evidence of a prothrombin conversion factor in plasma, distinct from L. F. or Ac-globulin. This entity is probably the inert precursor of spca to which it becomes converted during coagulation (14, 8). Like prothrombin, it is adsorbed by $BaSO_4$, and thus is probably identical with Quick's prothrombin A (earlier terminology) and perhaps with Milstone's prothrombokinase, which also is removed by $BaSO_4$ (21). Additional evidence for this factor was obtained by Landwehr and associates on a subject with congenital hypoprothrombinemia (22). When normal plasma was diluted with the prothrombinopenic plasma, the ensuing mixture exhibited considerably faster prothrombin conversion than a mixture of normal plasma with normal plasma deprothrombinated by $BaSO_4$ Furthermore, treatment of the prothrombinopenic plasma with this adsorbing agent obviated the accelerating effect, yielding a plasma indistinguishable from normal $BaSO_4$ plasma. Evidently, this patient's plasma contained a prothrombin-conversion substance adsorbable by $BaSO_4$ and not present in $BaSO_4$ normal plasma.

From the data of the present report it is concluded that our patient's clotting disturbance consisted in deficiency of this entity, the precursor of spca. We have seen that the pathological serum contained little spca after coagulation had run its course, and that normal serum or spca fractions rectified the retarded prothrombin activation. Furthermore, removal of spca rendered the serum inert.

Also in harmony with this interpretation are some of the other observations. Whereas normal or dicumarol plasma can partially correct the clotting defect, adsorption of the plasmas with $BaSO_4$ renders them relatively inert. This indicates that $BaSO_4$ removes the accessory factor in question. It also explains why the patient's plasma, assumed to be deficient in this entity, acted almost the same as $BaSO_4$ normal plasma as a diluent for normal plasma in the one-stage test. Furthermore, prothrombin preparations obtained from the abnormal plasma would be expected, as was found, to show the same clotting defect when mixed with $BaSO_4$ normal plasma.

Certain deductions may be drawn regarding the role of this clotting factor in prothrombin conversion. By the two-stage method, the rate of thrombin evolution as well as the final yield of thrombin from the patient's prothrombin appeared normal. Also, prothrombin consumption during spontaneous coagulation was unimpaired. Since under these conditions the prothrombin conversion process can be accurately followed except during the first few minutes after the addition or appearance of thromboplastin, it may be justifiably concluded that the clotting derangement lay in this phase of prothrombin activation, indeed, in the very interval represented by the elevated one-stage prothrombin time. It thus appears that the clotting factor, deficient in the pathological plasma, functions somehow in the early activation of prothrombin by thromboplastin plus calcium. Its similarity, in this regard also, to Milstone's prothrombokinase is striking (21).

The discrepancy in "prothrombin" values obtained on this patient by the one- and two-stage procedures raises questions regarding the validity of these methods, and their clinical diagnostic value. Obviously, the clotting disturbance would have escaped detection had the two-stage system been used exclusively. On the other hand, it is equally clear that the one-stage test, even the modification using $BaSO_4$ normal plasma as a diluent to assure adequate L. F., may fail to measure prothrombin specifically, since it is now apparent that $BaSO_4$ removes an important accessory substance. As has been emphasized elsewhere (9, 22, 23), unless due consideration is given to *all* the prothrombin-conversion factors as well as to the concentrations of fibrinogen, antithrombin and anticoagulants, translation of an observed prothrombin time into a specific prothrombin value may be fraught with serious error. Nevertheless, this case demonstrates the distinct value of the one-stage method in disclosing certain clotting abnormalities centered around the conversion of prothrombin to thrombin.

The clot-promoting effect of serum and purified spca is of practical as well as theoretical interest. To reduce the prothrombin time from 70 to 22 seconds is tantamount to increasing plasma prothrombic activity from about 3% to 25% of normal, a level which is consistent with effective hemostasis if other hemostatic mechanisms are normal (9). It has been shown that serum as well as purified spca can promote the coagulation of normal blood, hemophilic blood, thrombocytopenic blood, heparinized blood and blood exposed to siliconized surfaces (8). The therapeutic possibilities of serum were explored with good results by Crockett and colleagues (24) in a case of "idiopathic hypoprothrombinemia." It is noteworthy that in their subject, plasma seemed equally effective, the beneficial results of each lasting for 24 hours or more in contrast to the relatively shortlived effect of serum in our case. These authors attributed the clot-promoting property of serum to Ac-globulin, probably not being aware of the fact that one to two hour old *human* serum is practically devoid of Ac-globulin activity (15, 8).

The normal prothrombin consumption in our case is worthy of additional comment. Since poor prothrombin utilization characteristically occurs in hemophilia, thrombocytopenia, thrombasthenia and certain and other hemorrhagic states associated with defective coagulation, the prothrombin consumption test promises to become important in distinguishing hemorrhagic conditions, related to clotting abnormalities, from other forms of pathological bleeding. That our patient's defect was associated with normal prothrombin consumption is, therefore, particularly noteworthy. Furthermore, it renders untenable our earlier concept that spca elaboration is related to the amount and/or velocity of prothrombin conversion (6, 7). Also, it signifies the importance in normal hemostasis of the kinetics of thrombin elaboration rather than the overall amount of prothrombin converted.

The literature fails to reveal cases which completely simulate that presented in this report. Quick (19) described two brothers with elevated prothrombin times which he attributed to deficiency of "prothrombin A" (earlier terminology). It is of interest that these subjects also showed good prothrombin consumption. Unfortunately, they were not exhaustively studied by simultaneous one- and two-stage prothrombin determinations as well as by measurements of plasma Ac-globulin and serum spca. The condition was called "pseudohypoprothrombinemia," a term which we consider more suitably applicable to a group of disorders including Ac-globulin deficiency (parahemophilia) and the derangement represented by our case. Since in our subject the condition was evident at birth, it is most probably congenital in origin.

## SUMMARY

A clinical and laboratory study is presented of a four year old girl with a hemorrhagic diathesis present since birth. An elevated one-stage prothrombin time was the cardinal abnormality. Plasma prothrombin was qualitatively and quantitatively normal by the two-stage procedure, as were also plasma Ac-globulin and Labile Factor. Several other features distinguished this case from parahemophilia (Factor V deficiency). Also excluded were anticoagulants as well as possible abnormalities in the antithrombin mechanism or in the thrombin-fibrinogen interaction.

Prothrombin conversion was retarded in the early phase of prothrombin activation, but prothrombin consumption was normal, being virtually complete within 30 to 60 minutes after the blood was shed. The coagulation defect appeared to consist of deficiency of another prothrombin-conversion accessory factor which is present in normal or dicumarol plasma in limited amount, and which is adsorbable by $BaSO_4$. Since spca was decreased in the patient's serum, and since normal serum or purified spca rectified the clotting abnormality, it is concluded that the entity deficient in the pathological plasma is the precursor of spca. The disorder may be considered a form of pseudohypoprothrombinemia, alongside Ac-globulin deficiency (parahemophilia), and constitutes a hitherto unknown pathologic state added to the many already recognized hemorrhagic conditions related to clotting abnormalities of congenital origin.

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# PLASMA PROTEINS IN TOXEMIAS OF PREGNANCY

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(Submitted for publication December 26, 1950; accepted, April 2, 1951)

Numerous studies of the toxemias of late pregnancy have consistently demonstrated the association of pre-eclampsia and eclampsia with disturbances of protein metabolism indicated by derangements in the protein composition of the blood. Whether these changes in themselves cause the symptoms of toxemia or are secondary to the elusive "toxic" factor has not been determined. Many circumstances are known to provide the basis for protein deficiency within the human body, by increasing the tissue requirement for protein or by interfering with assimilation and utilization of an intake which would otherwise be adequate (1, 2). Evidence relating to the possible relationship of nutritional disturbances to the occurrence of toxemia is contradictory (3–9). In an excellent analysis of the problem of comparing conflicting reports in the literature, Møller-Christensen and Thygesen (10) emphasized the intimate connection between changes in protein balance and accompanying shifts in water and salt balances, and suggested the possibility of derangement in function of the adrenal in protein metabolism in toxemia. A complex explanation involving the presence of a toxic protein (an atypical euglobulin) in the circulating blood during menstruation, labor, and toxemia of pregnancy has been advanced by Smith and Smith (11, 12).

While a relationship between disturbed protein metabolism and toxemia of pregnancy has been established, the evidence does not show that the disturbance results from dietary protein deficiency. Dieckmann (13) believes that hypoproteinemia is neither the cause of pre-eclampsia and eclampsia, nor the cause of edema. Novak and Lustig (14) caution against identification of the hypoproteinemia of pregnancy with hypoproteinemia due to malnutrition and believe that within limits it is a physiologic condition. The finding by Bibb (15) that most women who developed toxemia had some previously existing hypoproteinemia would be expected. Despite suggestive data, theories relating toxemia to nutritional deficiency fail, as do all other hypotheses advanced, to explain many features of this disorder, notably its singular predilection for the primigravida. Our increased understanding in the past few years of the importance of interrelationships among various components of the food intake may provide a key to further elucidation of the role of nutrition in toxemias of pregnancy, but it seems likely that both nutritional and physiologic factors may be involved, the number varying perhaps with still other conditions. Certainly, even with respect to the part played by well-established changes in blood proteins, eclampsia remains "the disease of theories."

The conflicting reports in the literature and the recent development of the electrophoretic technique of analysis were responsible for the decision to determine the distribution of plasma proteins in the blood of women during pregnancy, at delivery, and in the puerperium. A preceding publication (16) reported the distribution of proteins in the plasma of healthy women during the menstrual cycle, during successive stages of normal pregnancies, and postpartum, demonstrating the shifts in plasma protein composition which are characteristic of uncomplicated gestation. The same techniques were employed under comparable conditions in investigations of the protein constituents of the blood plasma of women whose pregnancies were complicated by toxemia and other gestational disturbances. The results demonstrate some of the causes of the wide variations encountered in the literature and indicate profitable directions for further study. Subsequent papers will record results of determinations of plasma proteins in samples from subjects with renal and liver diseases, hypertension, diabetes, and other conditions.

## PROCEDURE

This report presents the results obtained from analyses of blood samples from 29 private and clinic patients admitted to the obstetrical services of Harper Hospital and Herman Kiefer Hospital, Detroit. All were studied first in the last trimester of pregnancy after toxemia had developed. The methods of obtaining blood samples, preparation, and analysis were identical with those described (16). The buffer solution (barbital) used was 0.1 N with respect to sodium diethylbarbiturate and 0.02 N with respect to diethylbarbituric acid. Electrophoretic recordings were made with a Tiselius instrument employing the Longsworth scanning mechanism and a three-piece Tiselius cell with a single, long center section. Determinations were run for three hours at 1° C. and a potential gradient of about 6 volts/cm.

Appraisal of history, physical, and laboratory findings, and postpartum outcome are usually necessary to permit some degree of accuracy in evaluation. Hence, final classification of the patients was determined only after postpartum observations were available. The patients studied fell into the following categories according to the

TABLE I

*Data from medical records of 29 patients with toxemias of pregnancy*

| | Subject* | Mother | | | | | | | | Infant | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Age (*yrs.*) | Parity/Gravida | Term gestation (*wks.*) | Blood pressure | | Albuminuria | Edema | Total weight gain during pregnancy (*lbs.*) | Sex | Weight (*gm.*) |
| | | | | | When admitted | Post-partum† | | | | | |
| Mild pre-eclampsia | 3 | 31 | 1/1 | 42 | 164/100 | 125/70 | + | ++ | 15 | M | 3005 |
| | 18 | 18 | 1/1 | 42 | 170/120 | 142/90 | + | 0 | 44 | F | 3288 |
| | 27 | 26 | 2/3 | 41 | 140/100 | 132/80 | + | ++ | 22 | M | 3827 |
| | 44 | 25 | 1/1 | 37 | 154/104 | 120/70 | +++ | + | 24 | M | 2282 |
| | 47 | 24 | 1/1 | 40 | 144/90 | 108/60 | ++ | + | 34 | F | 3714 |
| | 52‡ | 41 | 1/5 | 38 | 174/90 | 160/100 | ++ | + | 38 | F | 3118 |
| | 53 | 26 | 1/1 | 42 | 155/80 | 122/80 | ++ | + | 43 | M | 3487 |
| | 73 | 26 | 1/2 | 40 | 180/100 | 150/90 | ++ | + | 28 | F | 3856 |
| | 78 | 23 | 1/1 | 35 | 146/104 | 105/70 | +++ | ++ | 22 | F | 1502** |
| | 108 | 34 | 1/1 | 41 | 160/90 | 120/70 | +++ | + | 50 | F | 3062 |
| | 118 | 26 | 1/2 | 35 | 174/120 | 120/90 | +++ | ++ | 30 | F | 1474 |
| | 123 | 28 | 2/2 | 41 | 152/92 | 120/90 | + | ++ | 23.5 | F | 3799 |
| Severe pre-eclampsia | 4 | 19 | 1/1 | 39 | 184/100 | 112/78 | +++ | ++ | 37 | F | 3090 |
| | 17§ | 37 | 4/4 | 32 | 155/90 | 138/90 | +++ | ++ | 53 | M<br>M | 1502**<br>1361** |
| | 24‖ | 18 | 1/1 | 39 | 162/118 | 124/80 | + | +++ | 21 | M | 2268 |
| | 31‖ | 35 | 2/3 | 37 | 190/100 | 150/90 | +++ | ++ | 23 | F | 2070 |
| | 32 | 38 | 3/3 | 39 | 180/110 | 115/75 | +++ | 0 | 8 | F | 1928 |
| | 37 | 23 | 1/2 | 43 | 170/110 | 146/104 | +++ | +++ | 22 | F<br>M | 2041<br>2665 |
| | 87‖ | 20 | 1/1 | 38 | 190/120 | 130/88 | ++++ | +++ | 31 | F<br>M | 2948<br>2466 |
| | 94 | 29 | 1/1 | 35 | 180/120 | 138/90 | +++ | ++ | 10 | F | 992** |
| | 107‖ | 26 | 3/5 | 30 | 190/120 | 140/90 | ++ | +++ | — | F | 3175** |
| | 169 | 35 | 1/2 | 34 | 198/100 | 130/90 | ++++ | ++ | 20 | F | 1502 |
| | 170 | 41 | 2/4 | 38 | 190/110 | 145/95 | +++ | ++++ | 12.5 | F | 1602 |
| | 171 | 26 | 0/1 | 35 | 170/100 | 132/70 | +++ | +++ | 36 | M | 2211** |
| Eclampsia¶ | 2‖ | 18 | 1/1 | 38 | 180/120 | 160/110 | ++++ | ++ | 13 | F | 3260 |
| | 14 | 26 | 1/1 | 35 | 140/110 | 110/64 | ++ | + | 13 | M | 1502 |
| | 64‖ | 36 | 6/7 | 42 | 215/115 | 130/90 | ++++ | ++ | 60 | F | 3969 |
| | 82‖ | 23 | 1/1 | 33 | 160/100 | 130/80 | ++++ | ++++ | — | M | 2495 |
| | 111‖ | 18 | 1/1 | 35 | 160/110 | 122/70 | ++++ | ++ | 37 | M | 2098 |

* Subjects 4, 18, 31, 37, 107, 170, and 171 are negroes; subject 47 is Mongolian.

† Approximately six weeks.

‡ Four previous spontaneous abortions; stilbestrol therapy during pregnancy.

§ Rh negative—high titer.

‖ No prenatal care. Abruptio placentae occurred with subjects 24, 107, and 171. Subject 31 previously was hypertensive.

¶ Convulsions occurred three times with subject 2; twice with each of the other patients.

** Stillborn.

clinical classification proposed in 1939 by the American Committee on Maternal Health: mild pre-eclampsia, 12 cases; severe pre-eclampsia, 12 cases; eclampsia, five cases. Pertinent information from the clinical histories of the patients is summarized in Table I.

## RESULTS AND DISCUSSION

The means and ranges of concentrations of total protein and protein fractions in the plasma of blood obtained prepartum, at delivery, and postpartum are given in Table II. The results, insofar as can be determined, were not influenced by any form of therapy prior to the time of blood sampling.[1] The distribution of the total protein among the protein fractions and the albumin/globulin ratios are given in Table III. Values for women whose pregnancies were uncomplicated (normal) and for non-pregnant women (16) are included for comparison. Individual values for each woman will be presented in a subsequent report.

Numerous publications (10, 13, 14, 18–22) have given the quantities of proteins in the blood in normal and toxemic pregnancies, based for the most part on the results of various salt fractionation technics. All agree on the general pattern of the changes in the blood proteins, described as "dyscolloidosis" by Seitz (19) and Eufinger (20) although the degree of alteration in toxemia and the clinical significance of these changes are variously interpreted. All agree that a definite hypoproteinemia, greater than that observed in normal pregnancy, is found in the toxemias. Since the technics employed differ, and more especially, since there is no uniformity with which the various clinical types and degrees of toxemia are classified, protein values reported cannot be compared directly.

In the preceding paper (16) we compared electrophoretic results in normal pregnancies and postpartum with those for healthy non-pregnant women. The data showed that by six to 12 weeks postpartum (average, eight weeks) "the return of the plasma protein pattern toward normal is almost complete. . . ." If in healthy women whose pregnancies have been uncomplicated, the levels of blood proteins five to six days postpartum are significantly different from non-pregnant values and have not completely returned to normal levels by eight weeks postpartum, on the average, it seems evident that accurate control data for women who have had toxemias of pregnancy cannot be obtained from the same subjects sooner than eight or more weeks following delivery.

In pre-eclampsia and eclampsia mean concentrations (Table II) in plasma of total protein, albumin, beta and gamma globulins tended to be lower than comparable values in normal pregnancy. The other globulin fractions showed trends dissimilar to those of total protein, albumin, and gamma globulin, with a tendency for values to be higher than in normal pregnancy.

In normal pregnancy mean values for fibrinogen (phi globulin) in the third trimester were higher than the average for non-pregnant women, showed little change at delivery and five or six days postpartum, and declined to below non-pregnant levels by six to 12 weeks postpartum. In pre-eclampsia a similar trend occurred at a somewhat higher level, with levels six to 12 weeks postpartum approximating the average for non-pregnant women. In eclampsia, however, the means before and at delivery were higher than that of any other group, at one to five days postpartum the average still was high, and six to 12 weeks postpartum the mean was higher than that of non-pregnant women. Both chemical and electrophoretic determinations of fibrinogen have disclosed levels in toxemia even higher than the recognized increase in normal pregnancy. According to Dieckmann (13), a falling fibrin level in eclampsia implies a grave prognosis, and Dillon and Schmitz (23) reported two instances of fatal fibrinogenopenia in eclampsia. Among the cases of severe pre-eclampsia for which data are reported in this paper were three in which premature placental separation occurred. For these three patients, plasma fibrinogen levels were lower than those of patients in whom this complication did not occur. The levels, however, were not lower than mean values in uncomplicated pregnancy.

In a consideration of blood proteins in the toxemias of pregnancy *two* trends must be evaluated in relation to concomitant manifestations of disturbed physiologic function: their relation (*1*) to levels

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[1] In one patient, stilbestrol therapy as recommended by Smith (17) was employed in regularly increasing amounts (up to 125 mg. daily) from the seventh to the thirty-fifth week of gestation to prevent abortion. Despite continuous estrogen therapy and normal hormone excretion this patient developed mild pre-eclampsia, with electrophoretic patterns similar to those of other subjects with the same type of toxemia.

TABLE II

*Mean concentrations of protein fractions in plasma of women with toxemias of pregnancy*

Grams per 100 ml. of plasma

| | Interval from delivery (*days*) | Number | Albumin | | Globulin | | | | | | | | | | Total Protein | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Alpha$_1$ | | Alpha$_2$ | | Beta | | Phi | | Gamma | | | |
| | | | Mean | Range | Mean | Range | Mean | Range | Mean | Range | Mean | Range | Mean | Range | Mean | Range |
| *Prepartum* | | | | | | | | | | | | | | | | |
| Mild pre-eclampsia | 8–35 | 3 | 2.77 | 2.41–3.38 | 0.44 | 0.38–0.55 | 1.00 | 0.83–1.13 | 1.22 | 1.13–1.33 | 0.65 | 0.53–0.72 | 0.52 | 0.41–0.64 | 6.60 | 5.86–7.44 |
| Severe pre-eclampsia | 6–44 | 2 | 2.63 | 2.41–2.84 | 0.52 | 0.43–0.61 | 0.87 | 0.78–0.96 | 0.90 | 0.86–0.95 | 0.63 | 0.61–0.65 | 0.46 | 0.34–0.58 | 6.01 | 5.52–6.50 |
| Normal (third trimester) | 4–86 | 15 | 3.07 | 2.65–4.18 | 0.45 | 0.26–0.63 | 0.78 | 0.62–1.02 | 1.22 | 0.83–1.51 | 0.58 | 0.22–0.81 | 0.61 | 0.37–0.83 | 6.71 | 5.33–7.93 |
| Mild pre-eclampsia | 1–4 | 4 | 2.55 | 2.42–2.73 | 0.45 | 0.40–0.55 | 1.02 | 0.68–1.35 | 0.96 | 0.77–1.09 | 0.60 | 0.54–0.64 | 0.61 | 0.52–0.72 | 6.19 | 5.74–6.81 |
| Severe pre-eclampsia* | 1–5 | 4 | 1.84 | 1.46–2.40 | 0.61 | 0.43–1.08 | 1.26 | 0.66–1.87 | 0.72 | 0.57–1.00 | 0.60 | 0.49–0.76 | 0.49 | 0.28–0.72 | 5.52 | 4.85–6.75 |
| Eclampsia | 3 | 1 | 2.43 | | 0.50 | | 0.67 | | 1.31 | | 0.72 | | 0.49 | | 6.12 | |
| *Delivery* | | | | | | | | | | | | | | | | |
| Mild pre-eclampsia | | 8 | 2.97 | 2.48–3.80 | 0.48 | 0.34–0.72 | 1.15 | 0.79–1.82 | 1.04 | 0.57–1.69 | 0.64 | 0.49–0.89 | 0.60 | 0.41–0.97 | 6.88 | 6.07–8.64 |
| Severe pre-eclampsia | | 9 | 2.28 | 1.52–2.83 | 0.48 | 0.31–0.67 | 1.00 | 0.57–1.87 | 1.02 | 0.63–1.68 | 0.67 | 0.44–1.01 | 0.60 | 0.25–0.99 | 6.05 | 4.46–7.44 |
| Eclampsia | | 4 | 1.52 | 0.91–1.92 | 0.54 | 0.50–0.58 | 1.14 | 0.78–1.42 | 0.97 | 0.54–1.32 | 0.73 | 0.61–0.90 | 0.34 | 0.26–0.43 | 5.24 | 4.26–6.09 |
| Normal | | 17 | 3.09 | 2.35–3.77 | 0.44 | 0.32–0.58 | 0.85 | 0.53–1.34 | 1.27 | 1.04–1.64 | 0.61 | 0.32–0.82 | 0.58 | 0.28–0.91 | 6.84 | 6.20–8.37 |
| *Postpartum* | | | | | | | | | | | | | | | | |
| Mild pre-eclampsia | 4–12 | 8 | 2.68 | 2.27–3.04 | 0.72 | 0.54–1.13 | 1.08 | 0.89–1.28 | 1.10 | 0.91–1.26 | 0.75 | 0.54–1.01 | 0.65 | 0.42–1.42 | 6.98 | 5.83–8.27 |
| Severe pre-eclampsia | 6–11 | 7 | 2.19 | 1.66–2.55 | 0.66 | 0.49–0.83 | 1.16 | 0.84–1.61 | 1.11 | 0.85–1.55 | 0.67 | 0.47–0.85 | 0.61 | 0.45–0.97 | 6.40 | 5.26–7.42 |
| Eclampsia | 6–11 | 2 | 1.85 | 1.68–2.02 | 0.79 | 0.76–0.82 | 1.34 | 1.06–1.61 | 1.20 | 1.09–1.32 | 0.71 | 0.70–0.72 | 0.53 | 0.48–0.58 | 6.42 | 6.19–6.65 |
| Normal | 5–6 | 10 | 2.75 | 2.24–3.09 | 0.60 | 0.40–0.71 | 0.96 | 0.74–1.13 | 1.21 | 0.92–1.45 | 0.60 | 0.37–0.76 | 0.49 | 0.18–0.67 | 6.61 | 4.87–7.54 |
| Mild pre-eclampsia | 42–56 | 3 | 3.74 | 3.11–4.20 | 0.32 | 0.30–0.35 | 0.70 | 0.66–0.77 | 0.95 | 0.77–1.13 | 0.47 | 0.38–0.51 | 0.88 | 0.74–1.04 | 7.06 | 5.96–7.65 |
| Severe pre-eclampsia | 40–82 | 6 | 3.81 | 3.27–4.50 | 0.36 | 0.25–0.50 | 0.73 | 0.48–1.22 | 1.01 | 0.49–1.28 | 0.45 | 0.16–0.64 | 0.94 | 0.63–1.46 | 7.30 | 6.18–8.59 |
| Eclampsia | 48–69 | 3 | 4.10 | 3.97–4.32 | 0.37 | 0.35–0.38 | 0.85 | 0.79–0.88 | 0.97 | 0.90–1.07 | 0.51 | 0.43–0.61 | 1.05 | 0.82–1.19 | 7.85 | 7.79–7.96 |
| Normal | 42–84 | 13 | 3.95 | 2.96–4.61 | 0.41 | 0.27–0.59 | 0.64 | 0.46–0.80 | 1.02 | 0.70–1.24 | 0.42 | 0.30–0.61 | 0.75 | 0.25–1.08 | 7.19 | 5.33–8.02 |
| Non-pregnant women | | 12 | 4.39 | 3.88–4.91 | 0.34 | 0.25–0.47 | 0.64 | 0.51–0.83 | 0.95 | 0.72–1.19 | 0.48 | 0.37–0.57 | 0.91 | 0.74–1.08 | 7.71 | 6.86–8.90 |

* Includes values for three patients in whom abruptio placentae occurred and whose plasma fibrinogen levels are discussed in a paper which will appear soon in Harper Hospital Bulletin (Detroit).

*Mean concentrations of proteins in the plasma protein of women with toxemias of pregnancy*

|  | Interval from delivery (*days*) | Number | Albumin (*gm. per 100 gm. protein*) |  | Globulin (*gm. per 100 gm. protein*) |  |  |  |  |  |  |  |  |  | Albumin/globulin ratio |  |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
|  |  |  |  |  | Alpha$_1$ |  | Alpha$_2$ |  | Beta |  | Phi |  | Gamma |  |  |  |
|  |  |  | Mean | Range | Mean | Range | Mean | Range | Mean | Range | Mean | Range | Mean | Range | Mean | Range |
| *Prepartum* |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Mild pre-eclampsia | 8–35 | 3 | 42.0 | 38.6–45.5 | 6.7 | 5.1–9.4 | 15.1 | 14.1–16.3 | 18.5 | 16.0–20.3 | 9.8 | 9.0–11.0 | 7.9 | 7.0–8.6 | 0.72 | 0.63–0.83 |
| Severe pre-eclampsia | 6–44 | 2 | 43.7 | 43.7–43.7 | 8.7 | 7.7–9.4 | 14.5 | 14.2–14.8 | 15.0 | 13.2–17.3 | 10.5 | 10.0–11.0 | 7.6 | 6.1–8.9 | 0.78 | 0.78–0.78 |
| Normal (third trimester) | 4–86 | 15 | 45.8 | 38.9–52.7 | 6.7 | 4.2–9.1 | 11.6 | 9.0–16.3 | 18.2 | 15.5–22.4 | 8.6 | 3.9–11.8 | 9.1 | 6.4–12.2 | 0.84 | 0.64–1.11 |
| Mild pre-eclampsia | 1–4 | 4 | 41.2 | 38.6–44.3 | 7.3 | 6.7–8.8 | 16.5 | 11.5–21.6 | 15.5 | 12.3–18.1 | 9.7 | 8.7–10.8 | 9.8 | 9.0–10.6 | 0.70 | 0.63–0.80 |
| Severe pre-eclampsia | 1–5 | 4 | 33.3 | 29.2–35.8 | 11.1 | 6.4–21.5 | 22.8 | 12.0–28.8 | 13.0 | 10.0–18.3 | 10.9 | 9.9–12.2 | 8.9 | 5.7–13.2 | 0.50 | 0.41–0.56 |
| Eclampsia | 3 | 1 | 39.7 |  | 8.2 |  | 10.9 |  | 21.5 |  | 11.7 |  | 8.0 |  | 0.66 |  |
| *Delivery* |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Mild pre-eclampsia |  | 8 | 43.2 | 40.1–47.9 | 7.0 | 5.0–11.8 | 16.7 | 12.7–27.1 | 15.1 | 8.5–19.5 | 9.3 | 8.0–11.2 | 8.7 | 7.6–12.2 | 0.76 | 0.67–0.92 |
| Severe pre-eclampsia |  | 9 | 37.7 | 28.4–42.7 | 7.9 | 5.6–9.6 | 16.5 | 10.4–32.2 | 16.9 | 9.7–22.8 | 11.1 | 6.7–13.8 | 9.9 | 5.5–13.5 | 0.60 | 0.40–0.74 |
| Eclampsia |  | 4 | 29.0 | 21.4–32.1 | 10.3 | 9.0–12.1 | 21.8 | 14.7–33.1 | 18.5 | 12.8–21.7 | 13.9 | 11.6–15.0 | 6.5 | 5.2–7.3 | 0.41 | 0.27–0.47 |
| Normal |  | 17 | 45.2 | 37.0–49.0 | 6.4 | 5.0–8.4 | 12.4 | 7.9–16.0 | 18.6 | 16.2–22.7 | 8.9 | 4.6–12.9 | 8.5 | 4.4–13.0 | 0.82 | 0.59–0.96 |
| *Postpartum* |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Mild pre-eclampsia | 4–12 | 8 | 38.4 | 32.2–45.3 | 10.3 | 8.2–15.6 | 15.5 | 13.0–17.6 | 15.8 | 12.5–18.6 | 10.7 | 8.0–13.9 | 9.3 | 6.1–17.2 | 0.62 | 0.47–0.83 |
| Severe pre-eclampsia | 6–11 | 7 | 34.2 | 30.3–37.5 | 10.3 | 8.2–13.4 | 18.1 | 13.2–24.6 | 17.4 | 13.8–20.9 | 10.5 | 8.9–12.0 | 9.5 | 6.8–13.1 | 0.52 | 0.43–0.60 |
| Eclampsia | 6–11 | 2 | 28.8 | 25.2–32.6 | 12.3 | 11.4–13.2 | 20.9 | 17.1–24.2 | 18.7 | 17.6–19.8 | 11.1 | 10.6–11.7 | 8.2 | 7.8–8.8 | 0.40 | 0.34–0.48 |
| Normal | 5–6 | 10 | 41.6 | 38.6–46.0 | 9.1 | 7.8–10.2 | 14.5 | 10.7–16.0 | 18.3 | 16.3–20.0 | 9.1 | 6.8–11.2 | 7.4 | 3.8–9.2 | 0.71 | 0.63–0.85 |
| Mild pre-eclampsia | 43–56 | 3 | 53.0 | 51.0–55.6 | 4.5 | 3.9–5.1 | 9.9 | 9.0–11.0 | 13.5 | 12.7–14.8 | 6.6 | 6.3–6.7 | 12.5 | 11.4–13.6 | 1.13 | 1.04–1.25 |
| Severe pre-eclampsia | 40–82 | 6 | 52.2 | 47.8–56.2 | 4.9 | 3.5–6.5 | 10.0 | 7.1–19.1 | 13.8 | 7.7–17.7 | 6.2 | 2.6–8.0 | 12.9 | 9.1–18.0 | 1.09 | 0.92–1.28 |
| Eclampsia | 48–69 | 3 | 52.2 | 49.8–55.4 | 4.7 | 4.4–4.9 | 10.8 | 10.1–11.2 | 12.4 | 11.6–13.7 | 6.5 | 5.6–7.6 | 13.4 | 10.5–15.0 | 1.09 | 0.99–1.24 |
| Normal | 42–84 | 13 | 55.0 | 51.4–61.5 | 5.7 | 4.3–7.4 | 8.9 | 7.6–11.1 | 14.2 | 12.2–17.8 | 5.8 | 4.1–8.2 | 10.4 | 3.7–14.6 | 1.22 | 1.06–1.60 |
| Non-pregnant women |  | 12 | 57.0 | 53.7–60.9 | 4.4 | 3.6–5.6 | 8.3 | 7.2–9.6 | 12.3 | 10.2–13.5 | 6.2 | 5.2–7.2 | 11.8 | 10.1–13.3 | 1.32 | 1.16–1.56 |

normal pregnancy and (2) to levels on-pregnant women. Heretofore, ata have not been available which it comparison of the trends from pre- ecovery in eclampsia with the trends pregnancy or the comparison of trends concentrations in mild and severe pre- at different times in relation to de- th values in normal pregnancy and for nant women. Undoubtedly, in addition to ces in method of sampling and analysis the s lack of values for various intervals pre- ostpartum, both in normal and complicated ancies, has been responsible in large meas- or discrepant results in the literature. The essively severe clinical picture described as pre-eclampsia, severe pre-eclampsia, and mpsia suggests a progressive change in accom- ying internal conditions in the body. The data Tables II and III show that such parallelism es not hold true for all protein fractions and for l times in relation to delivery. Likewise, the pro- ein values accompanying increased severity of the clinical picture do not consistently increase or decrease in relation to comparable values for normal pregnant women.

From the data in Table II it is readily understandable that Lagercrantz (24) found a decline in total protein and albumin during the last month of gestation but with values no lower than those for normal pregnancy in his group of unassorted patients with various types and degrees of toxemia. Our study shows, on the contrary, little change in the mean quantities of total protein and albumin from the third trimester to delivery in normal pregnancy (Table II). Thus, with a mean of normal values within a lower range than that found in our study, or with a mean for toxemia which by chance represented subjects whose levels ranged higher for their degree of toxemia, it is understandable that similar or even higher than normal levels might be found in other series of toxic women. Thus, also, the possible lowering of total protein levels in eclampsia during the five days immediately prepartum would be obscured and as well, the trends we have observed toward lower concentration of total protein and albumin with increased severity of pre-eclampsia.

Whether the lowered plasma protein concentrations in toxemia actually represent a real loss of protein supplies in the body can be determined only by measurements of blood and plasma volume, for which precise methods are not available. Studies of blood volume in pregnancy (25) have consistently shown an increase of plasma volume of approximately 25 per cent subject, however, to great individual variations. In the evaluation of differences in protein concentration and distribution between women with and without toxemia the question is not whether blood volume increases during gestation but, rather, whether the increase varies when toxemia occurs. Since rapid changes in water balance occur in toxemia, deductions based on averages of a few values are especially subject to error and valid conclusions can be reached only with results for a large number of women or by following the same patients at frequent intervals, especially late in gestation and early in the puerperium. The data obtained in this investigation emphasize that confirmation or refutation of the numerous speculations which have been advanced will require more detailed study of accurately defined groups, both normal and abnormal, at more frequent intervals, and with comparable methods.

The data strongly suggest that the eclamptogenic syndrome is accompanied by alterations in the plasma proteins which represent a modification of changes which are physiologic in pregnancy. The deviations from normal in pre-eclampsia during the third trimester of pregnancy indicate that changes in the amounts and distribution of the plasma proteins may antedate the appearance of clinical signs and symptoms of toxemia. However, no evidence was obtained of a relationship to dietary protein inadequacy before or during pregnancy. The fact that the eclamptogenic effects in the third trimester, notably the increases in fibrinogen and the alpha$_2$ globulins and the decreases in gamma globulin and albumin, occurred on the basis of percentages of total protein as well as in terms of plasma demonstrates that the alterations are not reflections of changes in blood volume.

## SUMMARY

The electrophoretic method is being used to study the plasma protein partum, at delivery, and postpartum in the blood women whose pregnancies are normal, and the whose pregnancies are complicated by toxemia, renal disease, hypertension, diabetes and other gestational disturbances. Means and ranges for total protein, albumin, and alpha$_1$, alpha$_2$, beta, gamma, and phi globulins are presented for 29 women who had mild eclampsia, severe pre-eclampsia, or eclampsia before delivery.

The results stress the need for determinations at narrower and more sharply defined intervals prepartum and postpartum for larger groups of patients carefully grouped according to diagnostic criteria and according to the severity of clinical symptoms.

Determinations of plasma proteins for non-pregnant women and for normal pregnant women and patients with toxemia at intervals of a few days and six to 12 weeks postpartum emphasize that normal non-pregnant levels of plasma proteins are not achieved for several weeks after delivery and determinations as early as ten days or two weeks postpartum do not provide adequate "control" data.

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# THE MEASUREMENT OF C-REACTIVE PROTEIN IN HUMAN SERA. COMPARISON OF THE CLINICAL TESTS ON THE BASIS OF A QUANTITATIVE METHOD

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(Submitted for publication January 31, 1951; accepted, April 9, 1951)

C-reactive protein is a substance which appears in the blood in the course of a variety of disease processes. The existence of this protein in serum was first recognized as a result of its property of reacting to form a precipitate in the presence of the somatic C polysaccharide of Pneumococcus (1), and this reaction is responsible for the name which is at present used for the protein. Since the original description of the reaction, a number of papers have appeared concerning the purification and properties of this protein, and the range of diseases in which it is present in the blood (2–6).

The determination of C-reactive protein in the blood as a measure of the activity of the disease process in acute rheumatic fever has been described by Anderson and McCarty (7). In this instance, a specific rabbit antiserum to human C-reactive protein was employed to detect the presence of the protein in patients' sera by means of a precipitin test, and the results of this test were correlated with clinical and other laboratory data in a series of 45 patients with acute rheumatic fever. Like the erythrocyte sedimentation rate and the white blood cell count, the test for C-protein is not a diagnostic test for rheumatic fever, since it is positive in other infectious and non-infectious diseases. However, in this series of cases it appeared to be a more sensitive index of disease activity than any other that was available.

The antigen-antibody test for estimating C-reactive protein is extremely sensitive in detecting small amounts of the substance in serum, but the procedure used is at best only semi-quantitative. The results are recorded on the basis of the volume of precipitate formed, ranging from zero in the absence of reaction to ++++++ in the case of a maximal reaction. It is obvious that readings of this sort may not be the same when made by different observers, and furthermore different lots of antiserum vary in the amount of precipitable antibody which they contain. In the present study an attempt has been made to obtain a more quantitative estimate of the amount of C-protein present in various sera, and to correlate the quantitative data with the results of the precipitin reaction.

The general use of the C-protein precipitin test with rabbit antiserum as a clinical laboratory test in rheumatic fever is at present limited because of the difficulties involved in obtaining pure C-protein preparations in quantities which allow large scale production of rabbit antiserum. An alternative to the use of rabbit antiserum for the detection of the C-reactive protein in serum is the original precipitation reaction which employs the relatively easily prepared C polysaccharide of the Pneumococcus (8). The latter reaction is best performed as a ring test in which a dilute solution of the carbohydrate is carefully layered on the serum to be tested. A visible precipitate forms at the interface, and the density of the ring is roughly proportional to the amount of C-protein present in the serum. The reaction is never obtainable between C polysaccharide and normal serum. Many earlier determinations done in this laboratory indicated that the carbohydrate does not give a visible reaction in the presence of low concentrations of C-protein which are readily detectable with the antiserum. In the present study a comparison of the sensitivity of the two indicator systems, C carbohydrate and antiserum, is presented.

Because the capillary precipitin reaction between the C-protein of serum and the rabbit antiserum is only roughly quantitative, the desirability of determining the actual amount of protein responsible for the different titers of precipitin reaction given by patients' sera is evident. This determination has been undertaken in the present study.

## MATERIALS AND METHODS

*Rabbit antiserum to C-reactive protein.* The antiserum was obtained by injecting rabbits intravenously with a preparation of human C-protein purified by the method of MacLeod and Avery (3, 4). This preparation was almost completely free of normal human serum proteins. The antiserum reacted slightly with normal human serum, and the cross-reacting antibody was completely removed by adsorption with dried normal human serum protein.

*Precipitin test with antiserum.* The precipitin tests with antiserum were carried out in capillary tubes according to the procedure described by Anderson and McCarty (7). This method is based on the capillary precipitin technique for serologic typing of group A streptococci described by Swift, Wilson and Lancefield (9).

*Pneumococcal Cx polysaccharide.* This polysaccharide was prepared by rapid lysis and deproteinization of the pneumococcal cells, as recently described by Anderson and McCarty (10). It is identical with classical C polysaccharide in the sensitivity of its reaction with human C-reactive protein, and either one can be used to react with and remove the C-protein from serum.

The Cx carbohydrate used in this work was free of protein and nucleic acid, and the analysis of its nitrogen and phosphorus content agreed closely with elementary analyses previously done on other samples of the carbohydrate.

*Ring tests with Cx polysaccharide.* The reactions between serum and Cx polysaccharide were carried out in pointed precipitin tubes in which approximately 0.1 cc. of an 0.1 mg./cc. Cx carbohydrate solution in 0.85% NaCl was very carefully layered over 0.1 cc. of the serum being tested. Each determination was controlled with a tube containing the test serum layered with 0.85% NaCl in place of the carbohydrate. After incubation for one hour at 37° C. the tubes were inspected for the appearance of a ring of visible precipitate at the interface.

*Patients' sera.* The specimens of serum used in this study were from 200 serial weekly bleedings on 18 patients with acute rheumatic fever, and from 68 bleedings on 18 patients with viral hepatitis. The patients studied were under observation and treatment in the Hospital of The Rockefeller Institute for Medical Research.

*Quantitative measurement of C-reactive protein.* The quantitative method for the determination of C-reactive protein depends on the spectrophotometric estimation of the amount of protein removed from serum by the addition of Cx polysaccharide. The following procedure is employed.

All sera are cleared by centrifugation if necessary. Samples of 0.4 cc. of serum are mixed with 0.4 cc. of an 0.1 mg./cc. solution of Cx polysaccharide in 15 cc. pointed centrifuge tubes. After incubation for one to two hours, the tubes are refrigerated overnight. The precipitate is thrown down by centrifugation and is washed three times with 5 cc. volumes of 0.85% sodium chloride containing 0.01% calcium chloride. The presence of calcium ion is essential to prevent any resolution of the precipitate during the washing process. The washed precipitate is redissolved in 0.2 cc. of 0.1 M sodium citrate and the solution is placed in a quartz micro-cell for measurement of the ultraviolet adsorption at 280 m$\mu$. In most cases the precipitate dissolves completely to give a clear solution, and if particulate material is present it is necessary to clear the solution by centrifugation before testing. In the concentrations used, the Cx polysaccharide does not show measurable adsorption in the ultraviolet, and the optical density of the polysaccharide-protein solution at this wave length is proportional to the concentration of protein.

Two aspects of the validity of this quantitative procedure were checked by serological methods. First, although previous experience indicated that a single concentration of Cx polysaccharide (0.1 mg./cc.) could serve as the precipitant throughout the entire range of C-protein concentrations expected in sera, this was further checked by submitting the serum supernatants (after removal of the C-protein-polysaccharide precipitates) to the sensitive antibody test for C-protein. It was found that the 0.1 mg./cc. solution of polysaccharide removed practically all of the C-protein from sera containing the highest concentrations and showed no evidence of prozoning with sera containing smaller amounts. Secondly, it was important to determine whether the washed C-protein-polysaccharide precipitates contained appreciable quantities of serum proteins other than C-reactive protein. This point was investigated by testing the redissolved precipitate by the precipitin method with high-titered rabbit antiserum prepared against normal human serum. Only negative or trace reactions were obtained in these tests, indicating that normal serum proteins were probably not present in amounts sufficient to affect the spectrophotometric readings.

The results obtained by this quantitative procedure do not appear to be influenced by the presence of *antibody* to pneumococcal C polysaccharide. This antibody was shown by Heidelberger, MacLeod, Kaiser, and Robinson to be present in most human sera in significant amounts (11). The fact that antibody does not interfere with the measurement of C-reactive protein is probably best explained by the following considerations: In the quantitative measurement of C antibody, Heidelberger and his associates found it necessary to use long periods of refrigeration (eight days or longer) to obtain complete precipitation by the polysaccharide, while in the case of C-reactive protein refrigeration overnight is sufficient; and furthermore if antigen-antibody precipitates were formed in the course of the procedure described above, they would not redissolve in the citrate solution and would be removed by centrifugation before the spectrophotometric measurement was carried out.

*Standardization of the spectrophotometric measurement of C-reactive protein.* For the purpose of calculating the actual protein concentrations represented by the optical density measurements, a standard curve was prepared, using a large lot of purified C-reactive protein on which nitrogen analyses were carried out. The purified C-protein was obtained from 615 cc. of thoracentesis fluid from a patient with lymphosarcoma. Preliminary study of a sample of this fluid showed that the greater part of the C-protein which it contained was bound to phospholipid; and since the phosphorus and nitrogen contributed by the phospholipid would have influenced the quantitative results on elementary analysis of the purified protein, a de-

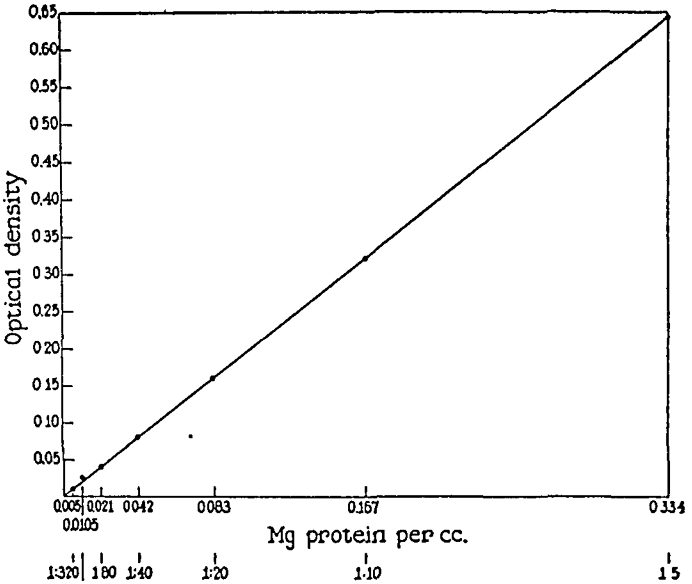

FIG. 1. THE STANDARD CURVE OF THE OPTICAL DENSITY READINGS ON THE BECKMAN SPECTROPHOTOMETER AT 280 Mμ OF DILUTIONS OF A SOLUTION CONTAINING A KNOWN AMOUNT OF PURIFIED C-REACTIVE PROTEIN

fatting procedure was used. The fluid was first fractionated with ammonium sulfate and the fraction which precipitated between 0.5% and 0.75% saturation was taken up in 68 cc. of distilled water. Following dialysis of the fraction against two changes of 1 M NaCl, the preparation was defatted with alcohol and ether in the cold, as described by MacLeod and Avery (3). The dried, defatted fraction after resolution in 200 cc. distilled water contained practically all of the C-reactive protein originally present in the fluid. This solution was then dialyzed against 0.01% calcium chloride to remove any lipid-bound C-reactive protein that might have escaped defatting. The dialyzed solution was cleared by centrifugation, brought to physiological salt concentration by the addition of molar saline, and the C-reactive protein was precipitated from the solution with 25 mg. of Cx polysaccharide. The protein-carbohydrate complex was washed five times in 0.85% NaCl containing 0.01% $CaCl_2$, and finally redissolved by adding a solution 0.1 M with respect to both sodium citrate and sodium chloride.

To obtain the values used in constructing the standard curve, the optical density of several carefully prepared serial dilutions of this final solution was measured at 280 mμ, and the protein nitrogen content of the material was determined. Since the Cx polysaccharide is a nitrogenous carbohydrate, it was necessary to correct for the nitrogen contributed by the polysaccharide. This was made possible by the fact that the polysaccharide contains phosphorus in addition to nitrogen, while C-protein does not. Thus, from the nitrogen and phosphorus analyses on the purified material and on the preparation of Cx polysaccharide used in the precipitation of the protein, it was possible to calculate with reasonable accuracy the amount of protein nitrogen present in the final material. It should be pointed out that the total amount of polysaccharide remaining in the final product was small in relation to the amount of protein and represented only a fraction of the 25 mg. initially used to precipitate the protein. Consequently, the correction required for polysaccharide nitrogen was not large. The phosphorus determinations were done by the King modification of the Fisk-Subbarow method, and the nitrogen determinations by the micro Kjeldahl technique.[1]

Figure 1 shows the relationship between the concentrations of C-reactive protein and its adsorption at 280 mμ. It has previously been determined that crystalline C-reactive protein contains 14.66% nitrogen (12), and the values for protein concentration are calculated from the nitrogen values on the basis of this figure.

## RESULTS

*Comparison of the sensitivity of the two clinical tests for measuring C-protein in patients' sera.* Consecutive weekly bleedings from 18 patients hospitalized for acute rheumatic fever were tested for the presence of C-reactive protein both by the precipitin test with specific antiserum, and by the ring test with Cx carbohydrate. The results obtained with both techniques on a total of 200 sera are shown in Table I.

It is evident from the data that the test system using the antiserum is definitely and consistently more sensitive than that in which the Cx carbohydrate is employed. The ring test with carbohydrate cannot be used as a reliable indicator for the detection of the C-protein in sera which give less than a ++± precipitin reaction with the antiserum. As shown by the quantitative data in Table IV, this indicates that a concentration of C-protein greater

TABLE I

*Comparison of the sensitivity of serum reactions of patients with rheumatic fever when tested with antiserum to human C-protein and with Cx polysaccharide*

| Precipitin reaction with antiserum | Number of sera tested | Number of positive reactions with Cx carbohydrate |
|---|---|---|
| +++ +++ | 7 | 7 |
| ++++ | 9 | 9 |
| +++ | 15 | 15 |
| ++± | 9 | 9 |
| ++ | 17 | 8 |
| +± | 8 | 2 |
| + | 42 | 1 |
| ± | 32 | 0 |
| trace | 23 | 0 |
| 0 | 38 | 0 |

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[1] The nitrogen analyses were made by Mr. Theodore Bella of The Rockefeller Institute and the phosphorus analyses by Dr. Lewis K. Dahl of the Hospital of The Rockefeller Institute.

than 0.05 mg./cc. is required to give a visible precipitate with the polysaccharide. Despite the failure of the carbohydrate to give visible rings of precipitate at the interface with sera containing less than the limiting concentartion of C-protein, it is apparent from the studies using the quantitative spectrophotometric technique that the reactive protein is chemically bound to the polysaccharide and can be removed by centrifugation.

In an attempt to increase the sensitivity of the carbohydrate ring test system, the carbohydrate solutions were made up in 1% crystalline bovine albumin solution and in 1% solution of human serum fraction V (13). It was found that the use of albumin intensified the ring with sera known to be reactive but did not cause the formation of visible reactions with sera that failed to give a positive ring test with the carbohydrate in saline.

From the point of view of clinical application, the lower sensitivity of the test with C polysaccharide greatly reduces its usefulness. This is unfortunate since the availability of C polysaccharide would make this procedure more feasible for general use than the test requiring specific antiserum. However, in the results described by Anderson and McCarty (7) and in subsequent studies in this laboratory, the determination of C-reactive protein in the evaluation of rheumatic activity is frequently most helpful in those cases in which the sera fall in the lower range of the precipitin reaction where the ring test with the polysaccharide is not reliable. In cases with more marked activity of the disease process, where both tests are strongly positive, other laboratory as well as clinical evidence of activity is usually available.

In addition to the studies on sera from rheumatic fever patients, precipitin tests have been carried out with sera from patients with a variety of other diseases, and in some instances it appears that the concentration of C-protein in the serum rarely, if ever, reaches levels that would be detectable with the C polysaccharide ring test. An interesting example is provided by studies on viral hepatitis. Havens, Eichman and Knowlton (14) have recently reported the failure to demonstrate the presence of C-protein by means of the C carbohydrate test in the sera of 90 patients with this disease. These workers called attention to the fact that the use of antiserum might have given different results, and this possibility has been investigated in this laboratory by testing 68 sera from 18 patients with viral hepatitis in the Hospital of The Rockefeller Institute. Thirty-eight of these sera gave positive precipitin reactions with the antiserum, but, as can be seen from the data presented in Table II, none of these reactions was of sufficient intensity so that a reaction with C carbohydrate would be expected. The incidence of positive precipitin reactions in relation to the duration of the disease is recorded in Table III, and it is obvious that the occurrence of C-protein in the serum is not limited to any particular phase of the disease. It appears that the occurrence of C-protein in the blood is a common, though not invariable, event in viral hepatitis.

TABLE II

*Magnitude of positive reactions between sera of patients with viral hepatitis and antiserum to human C-reactive protein*

| Precipitin reading | No. of sera |
|---|---|
| ++ | 6 |
| +± | 4 |
| + | 10 |
| ± | 10 |
| trace | 8 |
| 0 | 30 |

TABLE III

*Results of precipitin tests between C-protein antiserum and sera of patients with viral hepatitis*

| Days of disease | Number of sera tested | Number of positive precipitin reactions |
|---|---|---|
| 1– 7 | 5 | 4 |
| 7–14 | 11 | 8 |
| 14–21 | 11 | 6 |
| 21–28 | 11 | 7 |
| 28–60 | 21 | 12 |
| over 60 | 9 | 1 |

*Quantitative studies of the C-reactive protein in the serum of patients with acute rheumatic fever.* Quantitative determinations of the C-reactive protein were done on the sera from serial bleedings on six patients with acute rheumatic fever. The concentration of the protein was calculated from the optical density readings by the use of the standard curve and was expressed in terms of mg. of protein per cc. of serum.

In Table IV the relationship between the precipitin reactions and the results of the quantitative test is presented. The mean optical density observed in the quantitative test has been calculated for all sera giving each of the different readings in the precipitin test.

TABLE IV

*Correlation of the results of the precipitin test with the quantitative data on C-reactive protein in the sera of patients with rheumatic fever*

| Precipitin reaction | Quantitative data<br>Mean optical density | <br>Mean concentration of C-protein mg./cc. |
|---|---|---|
| +++ +++ | 0.616 | 0.330 |
| +++++ | 0.510 | 0.273 |
| ++++ | 0.430 | 0.230 |
| +++ | 0.235 | 0.126 |
| ++± | 0.208 | 0.101 |
| ++ | 0.101 | 0.054 |
| +± | 0.087 | 0.046 |
| + | 0.043 | 0.021 |
| ± | 0.027 | 0.010 |
| Trace | 0.013 | 0.006 |

As indicated by these data, 1 cc. of a serum giving ++++++ precipitin reaction contains over 0.3 mg. of this abnormal serum protein. Sera obtained from patients during the most active stage of acute rheumatic fever give marked reactions ranging from ++++ to ++++++ in precipitin tests with C-protein antiserum. On the basis of the quantitative data presented in Table IV, precipitin reactions of this order indicate that there is an appreciable total amount of C-protein in the circulating blood at the height of the disease. As recovery progresses this protein is present in decreasing amounts until it disappears from the blood completely, often before the erythrocyte sedimentation rate has returned to normal (7).

The quantitative fluctuations in C-reactive protein in the serum during the course of rheumatic fever are illustrated graphically by the charts of two patients given in Figures 2 and 3. Figure 2, on patient J. D., illustrates a simple monocyclic pattern of rheumatic fever with major manifestations of fever, polyarthritis, and carditis. The C-reactive protein in this patient showed a sharp uninterrupted drop to zero from the high level of 0.360 mg./cc. present during the most acute phase of the illness.

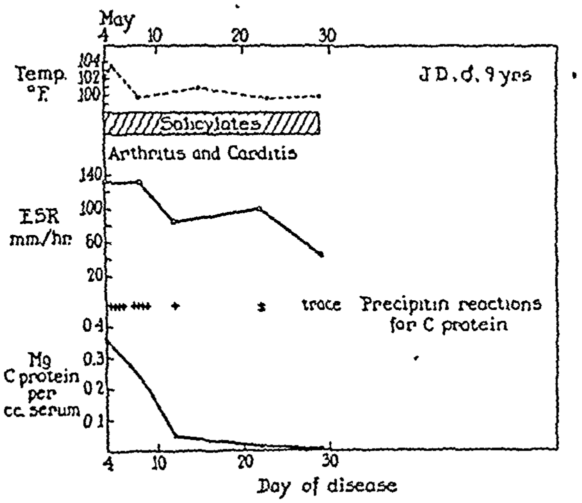

FIG. 2. PATIENT J. D. VARIATIONS OF C-PROTEIN IN RHEUMATIC FEVER

Correlation of C-protein determinations with other laboratory and clinical data.

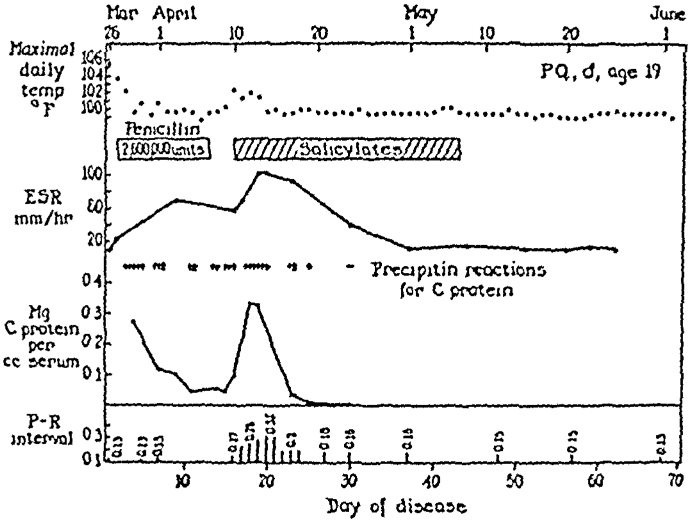

FIG. 3. PATIENT P. Q. VARIATIONS OF C-PROTEIN IN STREPTOCOCCAL PHARYNGITIS FOLLOWED BY RHEUMATIC FEVER

Correlation of C-protein determinations with other laboratory and clinical data.

In Figure 3 the variations of C-protein concentration in the blood are illustrated in a case of streptococcal pharyngitis which was followed by a recurrence of rheumatic fever. This patient, P. Q., a known rheumatic, was admitted to the Hospital of The Rockefeller Institute on March 26, 1950, at the first appearance of sore throat, malaise, and fever. A group A, type 18 streptococcus was cultured from this patient's throat and nose. The acute pharyngitis, which was paralleled by the appearance of a high level of C-protein (0.272 mg./cc.), was treated with penicillin and as clinical recovery occurred, the C-protein almost disappeared. However, on the 16th hospital day, the temperature and erythrocyte sedimentation rate both began to rise. These signs, along with clinical and electroacardiographic evidence of myocarditis, were accompanied by a rapid and marked increase in the amount of C-protein in the serum. As can be seen from the chart, the fall in concentration of C-protein reflected the subsidence of carditis, the only major manifestation of this patient's disease, more accurately than did the fall in the erythrocyte sedimentation rate. The biphasic nature of the curve of C-protein concentration is of interest with respect to the evaluation of the latent interval between the streptococcal infection and the onset of rheumatic fever. Although C-protein did not completely disappear from the blood in this instance, it had fallen sharply from 0.27 mg./cc. to 0.04 mg./cc. in seven days before the appearance of symptoms of rheumatic fever. This rate of fall is comparable to that seen in certain cases of uncomplicated streptococcal infection. It would appear from this and other data, therefore, that there is nothing in the pattern of C-protein response which makes it possible to predict the imminence of rheumatic fever as a complication of streptococcal disease.

## DISCUSSION

As a result of the use of the quantitative procedure for the estimation of C-reactive protein, it is now possible to give reasonably accurate values for the concentration of this substance in the blood during various disease states. While the spectrophotometric method is not suggested as a routine clinical procedure, it appears to have value in the study of certain cases of special interest in which more exact data concerning the fluctuations in concentration of C-protein are desirable. Moreover, the quantitative data allow a more precise comparison of the sensitivity of the two tests which have clinical applicability, and it is apparent that the test with specific antibody is greatly superior to that with the C polysaccharide. The ring test with C polysaccharide is not consistently positive at concentrations of C-protein below 0.1 mg./cc., while the antibody test will detect the presence of less than 0.01 mg./cc. In view of this fact, it is clear that at present the antibody procedure is the method of choice whenever the concentration of C-protein in the blood is to be used as a reflection of the activity of a disease process.

## SUMMARY AND CONCLUSION

1. A quantitative method for the determination of C-reactive protein in human serum is described. Although absent from normal serum, the protein has been shown to reach concentrations above 0.33 mg./cc. in acute rheumatic fever.

2. The C-reactive protein may be detected semiquantitatively by its reaction with the pneumococcal C polysaccharide or by a precipitin test with a specific rabbit antiserum. The sensitivities of these two methods have been compared and it is shown that the reaction with C polysaccharide is not reliable at serum C-protein concentrations below 0.1 mg./cc., while the rabbit antiserum gives positive reactions in the presence of less than 0.01 mg./cc. It is concluded that the C polysaccharide test cannot replace the antibody test in the clinical procedure in which C-protein is determined as a measure of rheumatic activity.

3. The sera of some patients with viral hepatitis contain C-reactive protein in concentrations high enough to be detected with the antiserum but too low to be detected by a visible reaction with the Cx polysaccharide. The presence of the C-reactive protein in sera from patients with viral hepatitis is not limited to the initial acute phase of the disease.

## ACKNOWLEDGMENT

The authors wish to acknowledge the able technical assistance of Miss Helen Babcock.

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# PROCEEDINGS OF THE FORTY-THIRD ANNUAL MEETING OF THE AMERICAN SOCIETY FOR CLINICAL INVESTIGATION HELD IN ATLANTIC CITY, N. J., APRIL 30, 1951

## Presidential Address

# AN INQUIRY INTO THE MEANING OF CLINICAL INVESTIGATION

By EUGENE B. FERRIS, JR.

Having approached emeritus status in the Society and being Editor of its journal for the past four years, it has not been possible to avoid considerable thought about the meaning of Clinical Investigation.

Frankly speaking, this inquiry has not led to sufficient insight for me to circumscribe its perimeters or to identify its characteristics from those of other sciences. The query has, however, raised some thoughts which I would like to share with you, since we are a society for Clinical Investigation and should have such awareness of its meaning as is consistent with its rapid and inevitable change in character.

I naturally went back to the origin of this Society and its constitution. The objects of the Society, as set down in the constitution, were most desirable but yet were not defined sufficiently for my purpose—so I perused the address of the first President, Dr. Meltzer (1).

It appears that in the beginning the founders themselves may not have been too clear as to what Clinical Investigation would turn out to be, but rather desired to encourage American physicians to make any kinds of objective and orderly observation on patients.

The first President seemed most concerned with a gangrenous disease of clinical medicine which manifested itself through a suicidal process by which each time a scientific growth developed in the field, it was extruded, like a foreign body, to become a special science—leaving clinical medicine to maintain a certain peace of mind, freed from the fetters of maturation into a science. It was by such a process, according to Dr. Meltzer, that the medical sciences, such as anatomy, physiology, biochemistry, etc., broke away.

One stated purpose of the founders was to bring back to clinical medicine those scientific disciplines which it had previously cast out so that one might say that Clinical Investigation represented an endogamous type of marriage between the allied sciences and the art of medicine, for as Dr. Meltzer stated in 1908: "I am of the opinion that clinical medicine as it exists now is made up of two constituents. One part has all the elements of a pure science and ought to be coordinate to the other pure sciences of medicine, and the other part is the real practice of medicine, an applied science which has many elements of an art." It appears that he was partial to the scientific mate of the union for he warned the members, "The constitution does not keep you down exclusively to science but let me tell you; beware of practice. It is a bewitching graveyard in which many a brain has been buried alive, with no other compensation than a guilded tombstone."

I think it can be said that Clinical Investigation has developed far along the pathways envisioned by the founders of this Society. In fact from some quarters one hears that the content of our program and that of our Journal is investigation all right, but bears only a slight resemblance to anything clinical. Perhaps it may be that Clinical Investigation has become somewhat Frankensteinian to some adherents of practice and perhaps also to some adherents of the "pure" sciences of medicine in the light of the competition which Clinical Investigation offers to the latter. For it now brings to bear in its study of man not only the disciplines of the biological sciences but those of the natural sciences as well and is beginning to encompass the social sciences.

It is of interest that the constitution requires that Society members have the M.D. degree and this requirement may have some symbolic connotation in defining, in a narrow sense, the peculiar differentiation of clinical from other forms of investigation, namely, the investigation of man as opposed to other forms of matter. Man, in health and disease and all things that affect his health, would seem to be the perimeter of the field. In order to study man, the clinical investigator must have both the training and the legal prerogative to do so in a responsible manner.

By and large Clinical Investigation has tended to apply all forms of investigative techniques and disciplines derived from other sciences to the study of man. It has been slow in developing that part which sets it aside from other fields of investigation, namely, the differentiating characteristics of man himself.

As a matter of fact, man is anathema to a very widespread principle of science, namely, the need to control in order to study. Man has been resisting control and seeking freedom throughout the ages, so that it is no wonder that he—and this includes the observer and the observed—has resisted the application of accepted scientific approaches. This factor has been ignored by many clinical investigators and deliberately bypassed by many others through the use of orderly experimental designs and statistical approaches, and through the correct use of the placebo—methods which can, and sometimes do, get the answer in spite of the fact that man is man.

The ability to investigate the more intricate workings of man requires some recognition of the facts that man resists being controlled, that he perceives through other

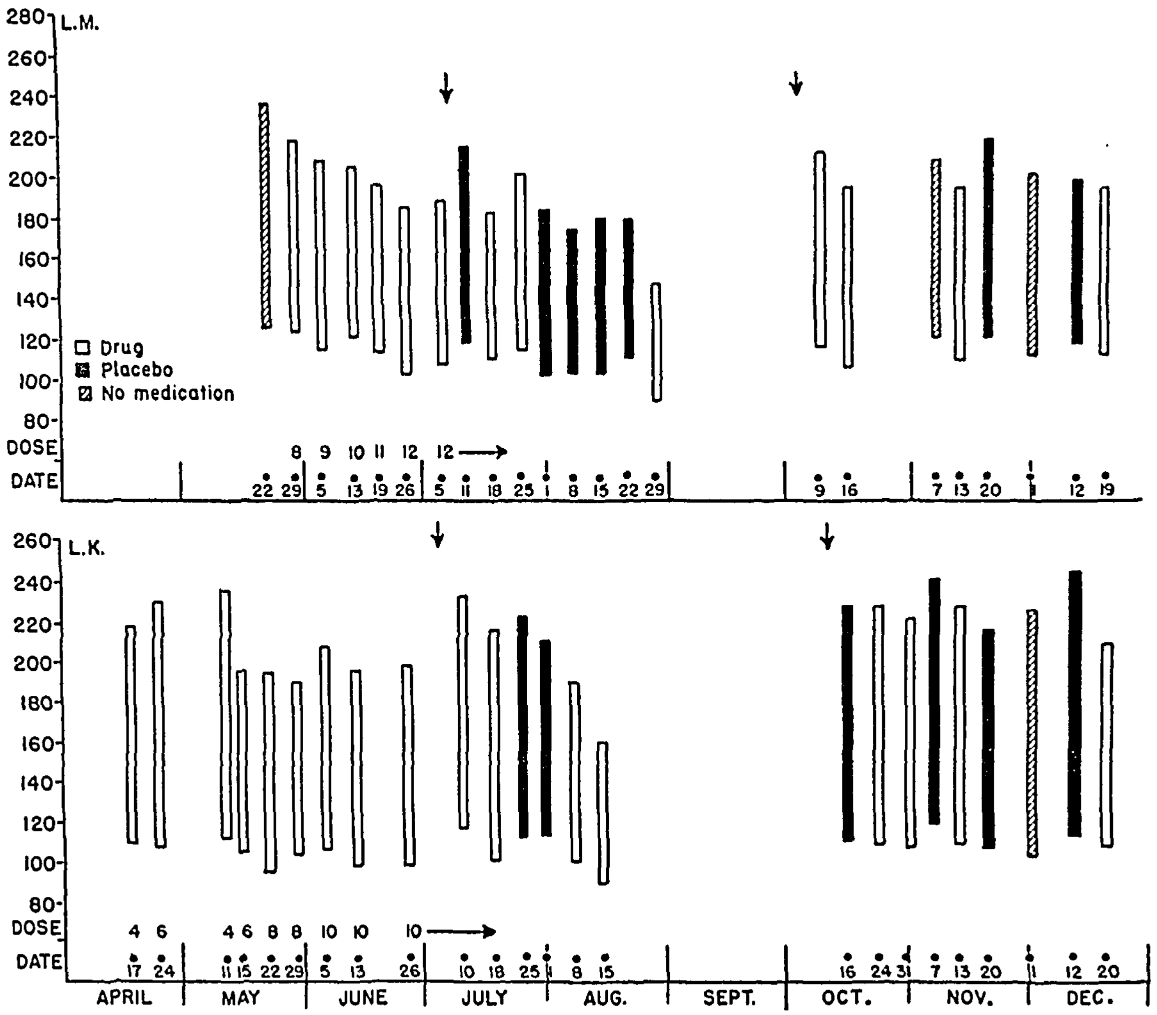

Fig. 1. The Effect of a Depressor Drug on the Stabilized Resting Blood Pressure of Two Patients Having Essential Hypertension

The dosage was built up to the indicated maximum and continued as drugs or placebo throughout the period of observation. Abscissa: BP in mm Hg; Ordinate: Dates as indicated. Bars represent systolic and diastolic limits of arterial pressure obtained by the auscultatory method.

than the five senses, that he communicates through the spoken and unspoken word, and that the investigator is just as human as the subject.

Thus we set up such conditions as the "basal state," "bed rest," etc., and may forget that people like bed rest or other forms of control to varying degrees. Some can relax without breakfast and others get angry at being denied it, so that controls may be more or less stressful depending on one's attitude toward them. It is known of course that feeling states, such as anxiety, can profoundly affect physiological homeostasis and that the placebo may be recognized by non-verbal communication.

The clinical investigator himself, through a need to control or dominate the patient, through his reaction to control or discipline of himself, and through his reaction to the success or failure of the patient to respond, may likewise affect the experimental result quite independent of his overt motives and desires or conscious integrity.

The following data from a group of studies to be subsequently reported serve as an example of the challenge to and special identity of Clinical Investigation as exemplified by the interaction of man, the investigator, with man, the investigated, and the intricacies which result therefrom.

Here are two women (Figure 1) in whom the depressor effects of a drug are being evaluated by one investigator-physician. As the dosage is raised to the point of optimum tolerance one notes a fall in blood pressure which is consistent with the hypothesis that it relates to the drug. It could also be related to the enthusiasm of the investigator and we know that, in this instance, there was such a feeling, so that it seemed appropriate to test the hypothesis further by the random substitution of blank pills for the drug. This procedure was acceptable to the investigator but it was more difficult for him to accept the further control that he, like the patient, should not know which was placebo and which was drug. In relation to the initiation of this control (July 9th), one notes an initial rise in blood pressure in the one case during blank administration and in the other case during the drug therapy, followed by a reduction in BP in both as the investigator accepted the new approach.

At this point, it is probable that as compared to the administration of blanks, the depressor effect of the drug is evident but certainly not world shaking. It is apparent that further observations of the effect of drug and blank are necessary in order to establish the statistical significance of the rather uninspiring depressor effect of the drug.

During September several phenomena occurred in the investigator which had in common a lessening of enthusiasm in the experiment but which were not known to the patients: (*1*) there was a lessened enthusiasm for the therapeutic efficacy of the drug and a resistance to continuing the experiment, (*2*) the investigator volunteered in the armed services for future assignment, and (*3*) he married and took a two weeks' vacation.

The experiment was then continued as planned, and it is quite evident that the blood pressure thereafter returned to approximate pre-treatment levels in which the drug itself still exerted perhaps a slight depressor effect. It seems quite possible that the patients may have sensed and responded to a change in the experimental atmosphere despite continued administration of pills.

This phenomenon of the interaction of the observer and the observed and the variable response of both to rigidly conceived methods of control, represents one phase of investigation which, if not peculiar to, is certainly a delineating characteristic of Clinical Investigation.

Any dynamic system is altered to some extent by the impact of a direct measurement, but man, particularly, responds also to observational techniques in which neither physical contact nor direct transfer of energy occurs. This latter response is inherent in Clinical Investigation. Though it cannot be avoided, other responses can be isolated from it by a sophisticated experimental design which recognizes that such an interaction is continuous between the observer and the observed, and that to the subject the Clinical Investigator is always cast in the mold of a physician.

As the intricacies of our techniques require more attention to fewer subjects, this interaction may become a dominant factor in the response. It thus becomes imperative that controls be applied in the positive sense of observing the truth rather than the negative sense of domination.

Clinical Investigation, as elusive as it is, has, I suspect, surpassed the fondest expectation of the founders when they started this Society 44 years ago. When it systematizes man's total behavior and weaves it into the galaxy of other scientific disciplines which it has applied so successfully to the study of man, there may be little to distinguish the art from the science of medicine.

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# ABSTRACTS

### *The Effect of Cortisone on Experimental Brucellosis.* Robert Abernathy, Minneapolis, Minn. (Introduced by Wesley W. Spink).

The basis of these studies was an attempt to resolve the problem of therapy in chronic brucellosis. The granuloma is the fundamental tissue response in brucellosis, simulating that found in tuberculosis. Brucella localize intracellularly, where the organisms survive and probably multiply. There is evidence that intracellular brucella are protected against the lethal action of antibiotics. Since the lesion of tuberculosis is altered by cortisone and tubercle bacilli disseminated, it was of importance to ascertain if a similar sequence took place in brucellosis.

Mice, guinea pigs and rabbits were infected with *Brucella abortus, Brucella mellitensis* or *Brucella suis*. Appropriate groups of animals with acute and chronic disease were treated with varying doses of cortisone administered once daily for 5 to 14 days. Serologic, cultural and histopathologic studies were carried out. Cortisone did alter the clinical course and tissue reaction of the infected animals. There was an increased death rate and the lesions were more widely distributed following the use of cortisone. The hepatic granuloma was converted into a necrotic lesion by cortisone therapy, which was most pronounced in infections due to *Brucella suis*. The agglutinin response was not influenced by cortisone. A discussion of the results will be accompanied by photomicrographs of the lesions.

### *Interrelations of Serum Lipids in Patients Treated with Cortisone and Pituitary Adrenocorticotropic Hormone (ACTH).* David Adlersberg, Stanley R. Drachman, Louis E. Schaefer and Rhoda Dritch, New York, N. Y. (Introduced by Ernst P. Boas).

Previous studies revealed well defined changes in the serum lipid partition of patients treated with cortisone or ACTH. These patients received hormonal therapy for a variety of pathological conditions, including disseminated lupus erythematosus, polyarteritis, rheumatoid arthritis, asthma, leukemia, scleroderma. Serial determinations included total and esterified serum cholesterol (Sperry-Schoenheimer method), serum phospholipid (Sperry modification of the Fiske-Subbarow method), serum total and neutral fat (Bloor method). The changes in the serum lipids may be, in general, summarized as a rise in the level of total and esterified serum cholesterol, often resulting in sustained hypercholesteremia with prolonged therapy; a gradual elevation in serum phospholipid roughly paralleling that of total serum cholesterol; a diphasic effect of serum neutral fat characterized by a transient and often precipitous fall during the first 2-3 weeks of treatment and later a gradual elevation above pre-treatment levels. Turbidity of "fasting" sera was observed, often at normal levels of serum neutral fat, probably indicating changes in the relative concentrations of serum lipid fractions or a change in the ratio of free and conjugated lipids (lipoproteins).

A study of the interrelationship of the serum lipid components appeared to be profitable. It included the following three ratios: Free/total cholesterol; total cholesterol/phospholipid; free/total cholesterol: phospholipid. Although the ratios noted before, during and after hormonal therapy fell as a rule within the normal range, there was a consistent alteration noted. A definite lowering of the free/total serum cholesterol ratio was observed in almost every instance. There was a less marked rise in the total cholesterol/phospholipid ratio, largely due to a disproportion in the elevation of total serum cholesterol to that of serum phospholipid. The ratio of free/total cholesterol: phospholipid revealed a marked and consistent reduction. It is suggested that a change in the rate of esterification of cholesterol and phospholipid synthesis may account for the observed alterations of the interrelationship of serum lipids. The significance of these observations will be discussed.

### *A Quantitative Approach to Separation of Lipid Mixtures.* Edward H. Ahrens, Jr. and Lyman C. Craig, New York, N. Y. (Introduced by Thomas M. Rivers).

Clinical investigations of lipid metabolism are hampered by lack of fundamental studies of chemical structure and physical properties of pure lipid compounds. Indeed, the incompleteness of basic data gives rise to many of the uncertainties of the methodology of the clinical laboratory. By their nature these methods can afford only approximate results: they are non-specific; calculations based on factors derived from studies of lipids in normal states may not be valid in abnormal states; certain lipid components can be estimated only by difference; the appearance of unusual compounds in disease may be completely overlooked. Primary difficulties in the organic chemistry laboratory have been produced by the strong association tendencies which hinder complete extraction of lipids and their subsequent separation into individual pure components. Attainment of single pure compounds is, however, essential to progress in this field. The present report will demonstrate that these forces can be overcome and lipid mixtures successfully separated by countercurrent distribution between immiscible solvents.

The countercurrent approach has made possible the quantitative separation of 5 grams of mixed lipids from one liter of human plasma into 3 main groups: phospholipids, cholesterol, and triglycerides plus cholesterol esters. The opportunity to characterize the composition of these cleanly separated groups and to enumerate and define their components is now presented for the first time. Fractionation of the third group is currently in progress, and will be followed by quantitation of the hydrolyzed fatty acids by distribution in another solvent system, an effective procedure developed as part of the present study. The initial group separation also demonstrated the presence of less than 25 mgm. of free fatty acids in the original mixture.

Experience with a wide variety of lipids permits the conclusion that countercurrent distribution, which is simultaneously analytical and preparative, presents the possibility of all-inclusive analysis of extremely complex mixtures of lipids.

### *Pseudohypoprothrombinemia and Parahemophilia: Deficiency of Different Components Essential for Rapid Prothrombin Conversion.* BENJAMIN ALEXANDER,* ROBERT GOLDSTEIN and GRETA LANDWEHR, Boston, Mass.

Recent recognition of non-prothrombin factors important in prothrombin conversion has permitted more precise delineation of the defect in certain patients who formerly would have been erroneously diagnosed as hypoprothrombinemia. Exhaustive study of these cases has disclosed hitherto unknown clotting components. The present observations indicate another plasma factor besides those already described. Its deficiency (or deranged function) causes retardation in the early activation of prothrombin despite normal prothrombin, Ac-globulin, Labile Factor and Factor V.

Two patients were studied with hemorrhagic diathesis and persistently elevated prothrombin times (70 and 60 seconds respectively) yet different coagulation defects. In the first, a 4 year old girl with hemorrhagic phenomena since birth, the clotting time, prothrombin consumption, plasma prothrombin, Ac-globulin, Labile Factor, and Factor V were normal, but serum prothrombin conversion accelerator (spca) elaboration was poor. Normal serum or purified spca rectified the abnormality in vitro and vivo. The disorder, hitherto unrecognized, is called "congenital hemorrhagic pseudohypoprothrombinemia."

In the other subject, a girl of 17 with bleeding episodes since 5, the abnormality was identical with Owren's case of parahemophilia. The following features distinguish this patient from the first: clotting time, elevated (42 min.); Factor V, Ac-globulin, Labile Factor activities were absent; prothrombin consumption poor; spca normal; prothrombin conversion retarded; corrected by normal plasma in vitro and vivo; serum and spca ineffective.

These contrasting features demonstrate convincingly the existence of two non-prothrombin plasma constituents important in thrombin evolution: (1) Factor V, which is unequivocally identical with Ac-globulin and Labile Factor; (2) the inert precursor of spca, which is activated during coagulation. Deficiency of either causes pathological bleeding and elevated prothrombin times simulating hypoprothrombinemia. Parahemophilia (Factor V deficiency) is benefited by *plasma*. Pseudohypoprothrombinemia (deficiency of spca precursor) is benefited by *serum*. The relative merits of the one- and two-stage prothrombin methods in distinguishing these disorders are apparent.

### *Toxoid Immunization in Clostridium Novyii Gas Gangrene.* W. A. ALTEMEIER,* C. L. COITH, W. R. CULBERTSON, M. A. LOGAN and A. A. TYTELL, Cincinnati, Ohio.

In previously reported experiments conducted jointly by the Department of Surgery and the Department of Biological Chemistry of the University of Cincinnati, under the auspices of the National Research Council and the United States Army, a Clostridium welchii toxoid was produced which developed a high degree of immunity in animals against a severe form of experimental gas gangrene caused by a highly virulent strain of Clostridium welchii.

Subsequent and unreported studies, under the same auspices, have yielded an effective Clostridium novyii toxoid which gives excellent immunity against severe infections in animals produced by the injections of 10 to 100 minimum lethal doses of virulent Clostridium novyii into closed wounds containing crushed muscle and dirt. Control animals which had not previously received injections of toxoid all died within 72 hours of a fulminating and extensive gas gangrene after the same challenge. Blood titers obtained in humans with this toxoid will also be given.

The unsettled international condition and the prospect of mass casualties predisposing to the occurrence of gas gangrene in the civilian as well as the military populations, makes this subject of timely importance and interest.

### *The Loss and the Retention of Potassium Which Occur Immediately After Starting and Stopping Adrenocorticotropic Hormone, Respectively.* FREDERIC C. BARTTER and PAUL FOURMAN, Boston, Mass. (Introduced by Fuller Albright).

The most constant metabolic changes which occur with adrenocorticotropic hormone (ACTH) administration in man are the transient K diuresis and retention which appear within 24 hours of starting and within 48 hours of stopping therapy, respectively. The present studies were undertaken to explore the mechanism underlying these changes.

The following points have been established: 1) The K changes are not due to contaminating antidiuretic hormone, since pitressin alone will not reproduce them; and they do not occur when the same preparations of ACTH are given to patients with unresponsive adrenals (adrenogenital syndrome). 2) The K loss is not due to catabolism, nor the K retention to anabolism of protoplasm, since the changes of K balance precede, and are relatively much greater than, those of N balance; furthermore, testosterone administered together with ACTH can prevent the changes of N but not those of K balance. 3) The K loss is not due to loss of normal intracellular fluid, since the loss precedes, and is relatively greater than, the loss of P. 4) The K retention is not a result of a non-specific, ACTH-induced K deficit alone, since it is not diminished by an increase in K intake before stopping ACTH. 5) The K changes are probably not secondary to reciprocal Na changes at the cellular level, since they are not modified by changes in the Na intake. 6) The urinary K loss precedes retention of Na and loss of P, electrical neutrality being achieved largely by a decrease of urinary acidity, an increase of urinary bicarbonate, or both. The urinary K retention precedes loss of Na, electrical neutrality being achieved largely by decreases of Cl and P.

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* Member.

### *Significant Remissions in Rheumatic Diseases with High Dosage Cortisone Therapy.* THEODORE B. BAYLES, FRANCIS L. COLPOYS, PAUL FREMONT-SMITH, BRUCE C. FERGUSON and EMIL PAIGE, Boston, Mass. (Introduced by Eugene C. Eppinger).

Standard dosage ACTH and Cortisone therapy in rheumatic diseases has failed to produce remissions after cessation of therapy. Ninety per cent of 135 patients so treated by us have relapsed within ten days after treatment. The experience of others is similar. Nine of our patients developing marked physiologic effects on ACTH therapy have experienced prolonged remissions. Accordingly, we have treated 12 patients with high dosage Cortisone, including seven cases of rheumatoid arthritis, one of rheumatoid spondylitis, one of juvenile rheumatoid arthritis, two of disseminated lupus erythematosus, and one of smoldering rheumatic fever with carditis. Previous standard dosage of ACTH or Cortisone in four of these patients had failed to produce lasting improvement.

Five hundred milligrams of Cortisone were administered intramuscularly daily for 14–28 day periods to the eleven adults, the juvenile rheumatoid arthritis patient receiving 300 milligrams daily. Clinical and laboratory improvement was noted in all cases. Transient psychotic episodes in two patients and marked fluid retention in a third were the only serious effects noted. These did not persist.

The first five consecutive patients treated have been followed for 74–88 days after threapy and all are in good clinical remission. In all cases, no evidence of continuing hormonal activity by the usual clinical and laboratory criteria and no evidence of continuing adrenal suppression could be demonstrated during the period of clinical remission after therapy.

Four patients have been followed for 13–32 day periods following treatment and are in good clinical remission. Three patients have just concluded therapy.

The prolonged remission in the first five patients and the shorter but definite remission in the next four so treated is highly significant in view of the previous high relapse rate on standard dosage. High dosage therapy can be administered with safety and deserves further investigation.

### *Clinical Observations on the Effects of Adenylic Acid and Cozymase.* WILLIAM B. BEAN,* Iowa City, Iowa.

Various adenine derivatives, including yeast- and muscle-adenylic acid and cozymase, have been injected intravenously into three classes of subjects: 1) convalescent hospitalized patients without cardiovascular disease, 2) patients who had experienced painful myocardial infarction in the past, and 3) patients with acute pellagrous glossitis. Control electrocardiograms were taken, using lead 2, and a continuous 2-minute tracing during and following injection with short runs 3, 5 and 10 minutes after injection was begun. All subjects experienced hyperpnea, a feeling of warmth throughout the body and occasionally abdominal pain and hyper-peristalsis. These usually disappeared within five minutes. Electrocardiographic changes were confined to T-waves and mechanism disturbance. Ninety-five per cent of patients had tachycardia after adenylic acid and about three-quarters showed T-wave changes. Similar changes occurred after cozymase was injected but they were not as common nor as extensive. Some patients who had had painful myocardial infarction in the past experienced sensations after adenylic acid resembling those they had with acute myocardial infarction.

Intravenous cozymase in doses of 100–300 mg. was found to produce a rapid improvement in pellagrous glossitis. This did not occur with yeast- or muscle-adenylic acid.

### *Studies on Acquired Resistance to the Shwartzman Phenomenon.* IVAN L. BENNETT, JR. and LEIGHTON E. CLUFF, Durham, N. C. (Introduced by Julian M. Ruffin).

It has been amply demonstrated that with repeated injections of the products of Gram-negative bacteria animals and human subjects rapidly acquire a tolerance for many of the toxic effects of these materials (fever, leukopenia, hypotension). This tolerance is of short duration, is not passively transferred by serum, and is non-specific in that tolerance to the "endotoxin" of one species applies also to similar substances from heterologous organisms. Many of these bacterial products are highly active in the production of the Shwartzman phenomenon. If the Shwartzman reaction is elicited repeatedly in rabbits, there rapidly develops a state of non-reactivity.

The experiments reported here were designed to determine: 1) if the production of typical skin reactions was necessary for the development of non-reactivity, 2) if incomplete reactions produced by giving only repeated skin or intravenous injections caused resistance, and 3) if thorotrast injection eliminated tolerance.

The materials used for the reaction were meningococcus filtrate or Prodigiosus polysaccharide (Shear).

Rabbits made resistant to typhoid vaccine consistently develop a state of non-reactivity to the Shwartzman phenomenon which disappears in 3–5 weeks if daily injections are discontinued. This material, in the doses employed, does not elicit the Shwartzman reaction.

Groups of animals were treated simultaneously as follows: A) given repeated Shwartzman reactions at 2 day intervals, B) receiving intravenous injections only, C) receiving skin preparatory injections only.

Animals in A developed complete resistance after 2–4 reactions of diminishing intensity. At this time, B and C animals uniformly showed complete tolerance to the reaction also. All animals exhibited a return of reactivity in 3–5 weeks.

Thorotrast injection promptly abolished tolerance in groups A, B, and C and also in animals treated with typhoid vaccine.

*Studies on Adrenocortical Eosinopenia.* WILLIAM R. BEST, ROBERT C. MUEHRCKE and ROBERT M. KARK,* Chicago, Ill.

We have described elsewhere the statistical errors and physiological variations in eosinophil counts of the circulating blood, and have demonstrated the importance of these factors in evaluating serial eosinophil counts. The present study is a clinical and statistical analysis of repeated four-hour eosinophil response tests to ACTH (Thorn test), epinephrine, ephedrine, and placebos in 135 healthy individuals and patients. The latter were ill with Addison's disease, malnutrition, cachexia, anorexia nervosa, proven pituitary or hypothalamic lesions, or other conditions. Several subjects were tested before and after splenectomy.

The majority show some eosinopenia following treatment with placebos (20% mean fall). A significantly greater eosinopenia (31% mean fall) is noted following ephedrine. The response to ephedrine is often much less than to ACTH in conditions bearing no known relationship to the pituitary-adrenal axis. On the other hand, significant eosinopenia was seen in patients with demonstrable lesions of the pituitary or hypothalamus.

The degree of response is independent of the initial eosinophil level. More consistency on repeated tests is noted in subjects with high initial counts and marked eosinopenic responses than in those with initial counts under 150/cmm. or with slight eosinopenic response.

From these studies we conclude: (1) Single tests with ACTH or ephedrine which fail to show significant eosinopenia do not establish the diagnosis of Addison's disease; (2) Marked eosinopenia indicates adequate adrenocortical function; (3) Repeated failure of response to epinephrine or ephedrine cannot be construed as indicating adrenal, pituitary, or hypothalamic disease; (4) Repeated failure of ACTH to produce eosinopenia strongly suggests nonfunctioning adrenals; (5) The level of response to epinephrine or ephedrine does not give accurate information regarding the integrity and functional activity of the pituitary-adrenal axis, and (6) The spleen does not play a part in the mechanism of adrenocortical eosinopenia.

*Observations on Survival of Mice After Exposure to Lethal Doses of X Radiation.* W. F. BETHARD and L. O. JACOBSON,* Chicago, Ill.

The discovery by Jacobson that spleen shielding in otherwise totally irradiated mice resulted in decreased mortality and more prompt hematopoietic recovery has been followed by a diligent search for the probable mechanism of action. Similar protection by cysteine, as shown by Patt, posed the question as to whether or not the two mechanisms were related. As a first step it seemed desirable to determine whether or not simultaneous use of the two methods would afford additive protection and for this an experiment was devised.

Approximate groups of mice (18 animals in each group) were subjected to 1100 r X radiation. Of the non-protected controls, all but one (95%) were dead within 28 days. Of those animals whose spleens were shielded with lead during irradiation, nine (50%) were dead in the 28 day period. A third group received 10 mg. of cysteine HCl intravenously 15 minutes before exposure to X radiation. Within the observation period 15 (83%) had succumbed. Animals of the fourth group received 10 mg. cysteine HCl as above. In addition, the spleen was lead-shielded during exposure to X radiation. Of these mice, only one out of eighteen (5%) died; and that death occurred on the first postoperative day.

Combined cysteine HCl administration and spleen protection yielded additive protection from X radiation over either method used singly. Further work is in progress to determine the LD50/28 days for mice given such protection.

*The Influence of pH on Blood Coagulation.* FREDERICK S. BIGELOW, Boston, Mass. (Introduced by Henry Jackson, Jr.).

Increase in alkalinity occurs in even briefly stored oxalated normal plasma, and has been found to cause errors in interpretation of certain coagulation tests. Within 2 hours during unstoppered storage at room temperature, the pH of 2 ml. samples rose to 8.4.

The thrombin-time of such stored plasma increased markedly. However, plasma samples, maintained at pH 7.4 by equilibration with mixtures of $CO_2$ and $O_2$ or $N_2$, showed unchanged thrombin-time for 6 hours at room temperature, and for 24 hours when frozen and prothrombin-free. Reduction of the pH from 8.4 to 7.4 shortened the thrombin-time to equal that of the equilibrated control. Thrombin-times were minimal in the pH range 6.25–8.60, at pH 7.30 in plasmas equilibrated with appropriate gas mixtures, and at pH 7.00 when adjusted by added 0.3N HCl or NaOH. The use of thrombin solutions buffered to pH 7.25 with imidazole failed effectively to control the pH of the reaction mixture.

Significant increases in recalcification-time of plasmas occurred within 1 hour of unstoppered storage. When kept at pH 7.4, there was but slight increase within the first 3 hours, but thereafter large increases occurred despite continued pH control. Systematic examination over pH range 6.25–8.8 revealed minimal recalcification times of 85–92 seconds between pH 6.2–7.5 with gas mixture equilibration and between 6.80–7.50 with HCl or NaOH adjustment.

Increase of the one-stage prothrombin-time occurred within 1 hour of unstoppered storage of mixtures of normal with prothrombin-free plasma. In equilibrated mixtures prothrombin-time was minimal at pH 7.35 and significantly lengthened at pH 8.00. Prothrombin-free plasma, after 72 hours in the frozen state at pH 7.40 did not retard coagulation when used as diluent in the one-stage procedure.

### *Myocardial Oxidative Metabolism and Initial Fiber Length of the Failing Human Heart.* R. J. Bing,* W. Falholt, R. Heimbecker and D. Carroll, Baltimore, Md.

Oxidative myocardial metabolism and cardiac efficiency were investigated in 80 patients with and without cardiac failure, by means of coronary sinus catheterization and the nitrous oxide method. The diastolic volume of the right ventricle was determined by injecting Evans Blue into the right ventricle through a double lumen catheter and suctioning the dye and blood mixture from the pulmonary artery through an automatic dye recorder. From the slope of the dye dilution curve, the residual and diastolic blood volumes of the right ventricle could be estimated.

In patients with increased diastolic volume but without myocardial failure, as in peripheral A–V fistulas, the myocardial oxygen consumption per unit weight was elevated. In patients with myocardial failure, the myocardial oxygen usage was normal despite large increases in the initial myocardial fiber length. These findings indicate that (a) failure is accompanied by a defective conversion of oxidative energy into useful work and that (b) an increase in initial tension of the heart muscle fiber augments oxidative myocardial metabolism only in the absence of chronic failure.

Cardiac glycosides caused a decrease in the initial fiber length and a rise in the work of the failing human heart. They had no effect on myocardial oxygen consumption. This suggests that cardiac glycosides act by influencing the initial fiber length and by a more effective conversion of oxidative energy into useful work.

### *Effect of Portacaval Anastomosis on Hepatic Oxygen Extraction and Estimated Hepatic Blood Flow in Patients with Cirrhosis.* S. E. Bradley,* A. I. MacPherson, A. Gammeltoft and A. H. Blakemore, New York, N. Y.

Measurements of hepatic oxygen extraction were made in 12 patients with portal hypertension due to cirrhosis before and after portacaval anastomosis. In 10 of these individuals hepatic blood flow (EHBF) could be estimated by the Bromsulfalein (BSP) clearance method. The hepatic arterio-venous oxygen difference increased in all but two. This value ranged from 3.0 to 5.3 vol. per cent preoperatively and increased by 16 to 110 per cent during the post-operative period (14 days to 30 months), ranging from 4.5 to 8.4 vol. per cent. Of the two patients showing a fall in hepatic oxygen extraction, one suffered from hepatic disease of such severity that BSP extraction was less than 10 per cent and the second was studied only 7 days after operation. It is possible that disturbances of hepatic function attributable to anesthesia and operation may have altered the response in the latter. EHBF fell in every case (from 10 to 110 per cent, 940 to 1870 ml. per min. pre-operatively and 530 to 1500 ml. per min. post-operatively). BSP extraction increased markedly in nearly every instance. These changes may be attributed to decreased portal venous inflow following the fall in portal venous pressure; the less rapid perfusion permitting more effective extraction of oxygen and BSP. Apparently an appreciable fraction of the oxygen supply to the liver is provided by the portal venous blood even in the presence of cirrhosis. This phenomenon may afford a means of assessing the potency of the shunt postoperatively.

### *Blood Pressure Responses to ACTH and Cortisone in Normotensive and Hypertensive Subjects in the Resting State and During Autonomic Blockade with Tetraethylammonium Chloride.* Albert A. Brust, William Ransohoff and Morton F. Reiser,* Cincinnati, Ohio.

Responses of the blood pressure and tetraethylammonium chloride (TEAC )"floor" to ACTH, cortisone, and desoxycorticosterone acetate have been studied. Appropriate metabolic changes were followed.

In six patients (3 hypertensive, 3 normotensive) who received ACTH, the "TEAC floor" rose above control levels within 24 hours after the drug was started and remained elevated throughout. Following discontinuance of the drug, "TEAC floors" fell below control levels. In three of the patients, changing the sodium intake produced additional shifts in the "TEAC floor" similar in direction and magnitude to the shifts induced by the same maneuver in the absence of adrenocortical stimulation.

Cortisone produced a slower but equally striking rise in "TEAC floor" in a normotensive and hypertensive patient, thus excluding pituitary pressor content of ACTH as the cause for the responses. With both drugs the usual depressor effects of TEAC were ultimately converted to a pressor rise, suggesting that autonomic blockade potentiates the vascular effects of ACTH and cortisone. DOCA did not produce a significant change in "TEAC floor" in a normotensive patient who responded dramatically to both ACTH and cortisone, ruling out DOCA-like action of cortisone in producing the observed vascular responses.

Resting blood pressures rose with both drugs, but the rise was less striking than that of the "TEAC floor." Blood pressure elevations were more prominent in normotensive than in hypertensive subjects.

The data suggest that: 1) Administration of ACTH or cortisone significantly alters the blood pressure and "TEAC floor." The responses are independent of sodium retention and indicate that the vascular effects of these drugs are mediated by a humoral mechanism which is potentiated by autonomic blockade. 2) The "TEAC floor" appears to be a sensitive indicator of adrenocortical activity as induced by ACTH and substituted for by cortisone. The responses precede eosinophil depression and other metabolic effects.

### *Elimination of Intravenous Carbon$^{14}$ Bicarbonate by Man.* Donald L. Buchanan, Chicago, Ill. (Introduced by Austin M. Brues).

A body of data has been obtained concerning the excretion rates and tissue retention of inorganic carbon$^{14}$ administered to rats and mice. The present study compares the human excretion of injected $NaHC^{14}O_3$ to rates observed in smaller animals in order to provide a basis for the computation of permissible human doses of this isotopic compound. In this work 10 microcuries of material was injected intravenously into healthy human volunteers and all expired $CO_2$ was collected in fractions during the following 3 hours. With the subject at rest approximately 75 per cent of the radioactivity is exhaled as $CO_2$ during the first hour; 90 per cent is gone in 2 hours; and 95 per cent has been blown off at the end of the third hour.

Within the error of the methods used the rate of isotope excretion by different sized animals is proportional to the metabolic rate per unit mass of animal. When published data of others are included this relationship seems valid for animals as large as the cow (Kleiber) and as small as the mouse. On this basis the extrapolation to man of information obtained in long term experiments with rats and mice indicates that an intravenous injection of 20–40 millicuries of $NaHC^{14}O_3$ or the inhalation of a comparable amount of $C^{14}O_2$ would be required to give a 70 kg man a total radiation dose of 1 roentgen equivalent over his entire life span.

### *The Determination of Traces of Calcium and Magnesium in Plasma.* EDWARD S. BUCKLEY, JR. and THERESA R. BORTOLOTTI, Boston, Mass. (Introduced by John G. Gibson, 2nd).

Currently available techniques are not sufficiently sensitive to assay calcium in plasma following its exposure to a cation exchange resin. No technique for the direct assay of plasma magnesium has been found satisfactory. Analytic techniques based on the principles outlined by Schwarzenbach have been developed. Murexide ammonium purpureate reacts with plasma calcium at pH values greater than 12.5 to give a red solution. Eriochromschwarz-T reacts with plasma magnesium at a pH of 10.8 forming a red color. Neither color obeys Beer's law and the murexide fades. If sufficient ethylene-diamine-tetra-acetate is added to either solution the metal-free color is obtained (purple for the murexide and green for the Eriochromschwarz). Ethylene-diamine-tetra-acetate reacts mole for mole with calcium and magnesium at these pH values, chelating all of the calcium before the magnesium. The calcium concentration can thus be computed from the murexide titration and the sum of the calcium and magnesium concentrations from the Eriochromschwarz titration. Potassium cyanide is included in the system to prevent interference from iron, zinc, and copper.

Using these techniques, plasma calcium concentrations ranging from 6.0 to 0.2 milli-equivalents per liter and magnesium concentrations from 2.5 to 0.10 milli-equivalents per liter have been measured.

### *Combined Antimicrobial Action Upon Penicillin Resistant Organisms* In Vitro. PAUL A. BUNN * and LEONARD CANARILI, Syracuse, N. Y.

Two micro-organisms recovered from patients with subacute bacterial endocarditis have been tested *in vitro* with a variety of antibacterial substances, singly and in combination. Both organisms were resistant to penicillin, 3 and 5 units penicillin respectively, and effective concentrations of streptomycin against each were 10 units. As they were difficult of eradication *in vivo*, studies were designed to demonstrate an interfering, or a synergistic action among the combination of other agents.

The two organisms, alpha hemolytic streptococcus and group D enterococcus, were tested in various dilutions and combinations of penicillin, streptomycin, terramycin, aureomycin, and chloramphenicol. Each organism was exposed to concentrations of at least two agents equal to, higher than and less than the measured sensitivity levels. Observations upon growth of organisms were noted at periodic intervals for 72 hours.

True interference of action of one antimicrobial agent against another was not observed. Synergism of high order occurred only with use of penicillin and streptomycin, each at levels of measured sensitivity. Reduced activity was noted with streptomycin at effective and penicillin at less than effective concentrations. When both were added in sub-effective levels, antibacterial action was less than when adequate amounts of either alone were used. With all other agents in combination with penicillin, neither an additive nor a synergistic effect was noted. Adequate and higher than adequate concentrations of each, when combined with the effective amounts of penicillin, resulted in more growth than when penicillin was used alone. Growth curves after aureomycin, chloramphenicol or terramycin with penicillin were identical and corresponded with those seen after sub-effective amounts of penicillin or streptomycin used singly. Use of three agents together did not reveal differences from that observed with two. Presence of penicillin always enhanced activity of aureomycin, chloramphenicol and terramycin.

### *Studies on Amethopterin, Citrovorum Factor, and Crude X-Methyl Folic Acid in Leukemia.* JOSEPH H. BURCHENAL * and E. M. KINGSLEY-PILLERS, New York, N. Y.

Amethopterin (4-amino-$N^{10}$-methyl pteroylglutamic acid) is chemotherapeutically active against several strains of mouse leukemia. From one of these sensitive parent strains of leukemia (AK4) a resistant mutant (AK4R) has been developed by successive passage through treated mice. This resistance is not due to a relatively increased content of folic acid (F.A.) or citrovorum factor (C.F.) in the AK4R cell, nor is it due to decreased adsorption or absorption of amethopterin by the resistant cell. AK4R spleen slices, incubated with amethopterin, show no increased ability to detoxify the drug. AK4R shows cross resistance to all 4-amino derivatives of F.A. tested, but not to 9-methyl folic, crude X-methyl folic, or 2,6 diaminopurine. The most likely explanations for this resistance would seem to be the development of an alternate metabolic pathway bypassing C.F. or a qualitative change in the enzyme for which both C.F. and amethopterin are competing so that there is a decrease in the affinity for amethopterin.

Since these data suggest that the crude antagonist of folic acid might be effective in patients that have become resistant to amethopterin, and that the folic-citrovorum-amethopterin relationship may be altered in the development of resistance, clinical studies on the effects of the crude antagonist and on the effects of massive doses of amethopterin buffered by C.F. have been undertaken.

In one patient who, on 2.5 mg. of amethopterin daily for eight days, developed toxic mouth ulcerations and leukopenia, doses of 45–60 mg. daily for twenty-one days were tolerated when covered by simultaneous parenteral administration of 3 mg. of C.F. No more beneficial effects were noted on the leukemia than from 2.5 mg. of amethopterin alone. Titrations of the competitive citrovorum-amethopterin antagonism and the effects of crude antagonist in patients will be reported.

### *Studies on the Nature of the Adrenal Cortical Secretion in Patients with Malignancy.* ROBERT B. BURTON, ALEJANDRO ZAFFARONI, ANTHONY J. IZZO and E. HENRY KEUTMANN, Rochester, N. Y. (Introduced by Lawrence E. Young).

The analysis of urine extracts by paper chromatography is so designed that each compound present having the chemical characteristics of the known corticoids can be isolated and its quantity estimated.

Study of the free urinary corticoids in patients with leukemia or lymphomas showed that with ACTH stimulation the steroid showing the greatest increase was Compound F. The excretion of this hormone rose ten to sixty times that seen in the pre-ACTH period. While Compound E was present in amounts equal to, or slightly greater than, Compound F in the pre-ACTH period, its concentration rose only three to ten times under therapy. The same response held true for a third steroid component whose identity will be discussed.

Untreated but well nourished patients with malignancy showed no significant difference from normal controls with regard to excretion of these major components. In cachectic patients with malignant disease the excretion of all components was moderately reduced.

In both normal subjects and in patients treated with ACTH a much larger quantity of each corticoid was excreted as glucuronide conjugate than as free corticoid.

### *Studies on the High Output Cardiac Failure of Occidental Beriberi.* JAMES A. CAMPBELL, LOUIS A. SELVERSTONE and DANIEL L. DONOVAN, Chicago, Ill. (Introduced by S. Howard Armstrong, Jr.).

To study high output cardiac failure and to compare it with failure of the low output type, hemodynamic measurements were obtained on 2 patients in cardiac decompensation with biochemically proved beriberi. The patients were young alcoholics without underlying disease who entered with cardiac enlargement, pulmonary congestion, peripheral edema, glossitis, and peripheral neuritis. Avitaminosis was demonstrated by thiamine excretion and metabolic load tests.

Prior to therapy these patients showed elevated resting cardiac indices of 4.3 and 7.2 L/min/M$^2$, which remained fixed during mild exercise. During this stress the stroke volumes fell, and pulmonary artery pressures rose markedly. These responses parallel from a high initial level those seen in a series of patients with low output failure, and indicate cardiac incompetence.

Following effective therapy with clinical restoration of cardiac competence, the resting cardiac indices in these patients dropped to 2.5 and 2.7 L/min/M$^2$ respectively with concomitant decreases in resting stroke volumes and pulmonary artery pressures. However, during exercise they were then able to increase their cardiac outputs and stroke volumes appreciably, while pulmonary artery pressures rose only moderately. Their responses had become similar to those seen in normal subjects studied in this laboratory. In one patient, coronary sinus catheterization was also performed before and after therapy, and blood oxygen, glucose, lactic acid, and pyruvic acid levels were measured.

Our data again show that high cardiac outputs occur in beriberi heart failure and decrease toward normal with the patient's clinical improvement. During decompensation the high resting values appear to be maximal; recovery is characterized by renewed ability to increase cardiac output during stress. The analogy with the hemodynamics of low output failure is drawn.

### *Effects of Paritol on the Prothrombin Times, Anti-Thrombin Times, and Lee-White Clotting Times.* DON W. CHAPMAN and ALAN A. ORY, Houston, Texas (Introduced by James A. Green).

Observations as to the effect of Paritol C on antithrombin and prothrombin activity and clotting times were made, in addition to antidote studies.

In 23 dogs, Paritol, administered intravenously in doses of 3, 5, and 7 mg/kg, produced a peak effect in 15 to 30 minutes with a prolongation of clotting times 2–3 times the normal value for 4–5 hours, and prothrombin time was notably prolonged. Protamine sulphate effects a prompt return of the clotting time to normal in a dose of 5 mg/kg. Toluidine blue was found to be unreliable.

Twenty-three patients, including 9 with acute myocardial infarctions, 4 with acute thrombophlebitis, 3 with arteriosclerotic heart disease, and 7 normals, have been treated with Paritol C administered intravenously for 24 to 72 hours, with an average of 50 hours without any demonstrable toxic effects or developments of clinical thrombosis or emboli.

Patients initially received a dose of 5 mg/kg of Paritol C intravenously and 3–5 mg/kg when the clotting time fell below 20 minutes. The clotting times were effectively prolonged to 10 hours with extremes of 6½ to 18 hours. This onset of action is within 10–15 minutes after its administration. Antithrombin activity was greatly increased immediately following the administration of Paritol C and fairly closely paralleled the clotting times. Dicumarol alone had no effect on the antithrombin activity in 14 patients. Serial prothrombin level determinations revealed an almost immediate prolongation in 8 of 12 cases tested with a maximum peak in 3–4 hours. In 2 the prothrombin levels dropped from 90 to 10 percent of normal without dicumarol.

When dicumarol and Paritol C are administered in combination, the antithrombin activity may enable one to differentiate their relative effect on the prothrombin time. When the prothrombin time is prolonged in the presence of increased antithrombin activity, Paritol C, in our opinion, may be partially responsible for its prolongation.

Because Paritol is an immediately acting anticoagulant of relatively low toxicity which has a more prolonged action than heparin, and is potentially economically more available, it may prove to be superior to heparin.

*Effect of Sub-total Adrenalectomy on Renal Hemodynamics and Electrolyte Excretion in Human Hypertension.* John Kapp Clark, Archer P. Crosley, Jr. and Harold G. Barker, Philadelphia, Pa. (Introduced by Francis C. Wood).

Measurements of renal hemodynamics and electrolyte excretion have been made before and after sub-total adrenalectomy with removal of approximately 90 per cent of adrenal tissue in 6 severely hypertensive patients. The change in glomerular filtration rate (inulin or endogenous creatinine clearance) was small and variable. In 5 of 6 patients effective renal blood flow (para-amino-hippurate (PAH) clearance and hematocrit) increased at a time when mean arterial blood pressure was decreased, thus indicating a reduction in overall renal vascular resistance. Tabular maximal excretory capacity (Tm PAH) was not consistently changed post-operatively. The characteristic pathological functional pattern of the hypertensive kidney consists of increased vascular resistance located chiefly in the efferent arteriole with resultant high filtration fraction and ischemia relative to the mass of perfused tissue. The data suggest that this pattern may tend to be reversed by sub-total adrenalectomy since the high pre-operative filtration fractions fell in all 6 patients and the ratio of effective renal plasma flow to Tm PAH increased in 3 out of 4 patients.

The rates of sodium, potassium and chloride excretion have been measured and correlated with the hemodynamic observations both pre- and post-operatively. The data show no systematic change in electrolyte excretion following surgery. This may indicate that enough adrenal tissue remained to prevent the electrolyte imbalance characteristic of Addison's disease, but the results are so variable, possibly due to changes in diet, rate of diuresis and state of hydration as well as to differences in the amount of gland still functioning that no conclusions can be drawn as to the effect of the operation on electrolyte excretion on the basis of these few cases.

✓*Observations on the Metabolic and Anti-Inflammatory Effects of Desoxycorticosterone, Testosterone and Cortisone in a Patient with Rheumatoid Arthritis.* William S. Clark, Elizabeth L. Manning and Janet E. Appleton, Boston, Mass. (Introduced by Walter Bauer).

Mineral and nitrogen balances were studied for 250 days in a 43 year old male with typical rheumatoid arthritis to determine the metabolic and inflammatory effects of cortisone, desoxycorticosterone and testosterone (in doses of 150, 25 and 100 milligrams per day, respectively), singly, together and in binary combinations for periods of 8 days. Sodium, potassium, calcium, chloride, phosphorus and nitrogen were determined. The actual and derived data revealed no metabolic anomalies during control periods. The administration of ACTH resulted in the expected loss of potassium, nitrogen, phosphorus and calcium with sodium and chloride retention. All three steroids resulted in sodium and chloride retention, however administered. All treatment combinations involving testosterone resulted in potassium, nitrogen and phosphorus retention. Desoxycorticosterone and cortisone caused potassium loss which increased when the hormones were administered together. Although cortisone and desoxycorticosterone did not appreciably alter nitrogen and phosphorus excretion, cortisone counteracted the nitrogen and phosphorus-retaining effects of testosterone. Cortisone increased calcium excretion, whereas testosterone decreased it; however, the latter effect was counteracted by cortisone. Desoxycorticosterone increased urinary calcium when it was administered with cortisone or testosterone and cortisone. Desoxycorticosterone prevented the calcium-retaining effect of testosterone. The data add further evidence of the antagonistic effects of testosterone and cortisone on nitrogen, potassium and phosphorus balances. They reveal an effect of desoxycorticosterone on calcium excretion and no antagonism between desoxycorticosterone and cortisone in relation to sodium and chloride excretion. They further suggest that the ACTH calcium diuresis may indicate an electrolyte hormone effect. They also reveal that the anti-inflammatory action of cortisone is not altered by testosterone and/or desoxycorticosterone and that this effect cannot be related to protein and electrolyte metabolism as measured by mineral and nitrogen balances.

*Enzymatically Hydrolyzable Formaldehydogenic Corticoids: Normal Values and Observations on Disease States.* A. C. Corcoran,* Harriet P. Dustan and Irvine H. Page, Cleveland, Ohio.

Treatment with beta-glucuronidase has been shown to increase yields of urinary corticoids. The present study deals with development and application of a standardized method for determination of total hydrolyzable formaldehydogenic corticoids (THFC) in normal subjects and in hypertensive disease; it includes observations in adrenal dysfunctions and on the effect of ACTH and cortisone.

Determinations in normal subjects yielded the following values in mg. per 24 hours: Males, mean $23.5\,\sigma \pm 16$; females, $16.6\,\sigma \pm 9.3$. Corticoid determined without enzymatic hydrolysis (FC) shows some correlation with corticoid glucuronide; the ratio FC/THFC averages 18.3 σ ± 8.8.

The mechanism of the wide variations in values is not apparent since such occurs in the same person. However, amounts of THFC are found to increase with urine volume (correlation coefficients, 0.53 in males and 0.71 in females). This is not due to effects of urine on activity of the enzyme. It is therefore attributable to some renal mechanism. This view is supported by the observation that total acid-hydrolyzable urinary glucuronide ("glucuronic acid") shows a similar association.

In essential hypertension we can demonstrate neither a sex difference in mean rates of excretion of THFC nor a correlation with urine volume. THFC values found in these patients were: mean 16 σ ± 8.9 in males and 15.3 σ ± 8.5 in females. As previously surmized, the ratio FC/THFC is sometimes very low, because high levels of FC occur in hypertensive disease.

Cortisone (100 mg. daily) does not increase yields of THFC. Preliminary observations in systemic lupus erythematosus in acute relapse, under treatment with massive doses of cortisone or ACTH, indicate that yields of THFC increase as the process comes under control. Destruction of the steroid side chain probably decreases as the disease abates. These analyses may become rational guides to dosage schedules and may prevent hypercorticoidism as a complication of hormonal treatment.

In Vivo *Studies of the Role of the Kidney in Intermediary Metabolism in Man and Dog.* James W. Craig, Max Miller,* William R. Drucker, Hiram Woodward, Bernard L. Brofman and Walter H. Pritchard, Cleveland, Ohio.

Renal excretory function has been emphasized in most studies of the normal and diseased kidney. However, there is evidence that this organ also plays a significant role in intermediary metabolism. The following studies of renal metabolic activities were therefore undertaken in man and intact anesthetized dogs.

The concentrations of glucose, pyruvic acid, citric acid, and malic acid were determined in urine and in samples of blood obtained simultaneously from the renal vein (by catheter) and femoral artery. Renal blood flow was measured by PAH extraction, and glomerular filtration rate by inulin (in man) and creatinine (in dog) clearances. Renal balances were calculated by the equation:

$$\text{Renal balance (mgm./min.)} = (\text{A}-\text{V})\ \text{RBF} - \text{U}$$

where A and V = concentrations (mgm./100 cc.) in femoral artery and renal vein blood respectively.

RBF = Renal blood flow (cc./min.)
U = Urinary excretion (mgm./min.)

In the fasting resting state small positive balances of glucose and citric acid were found.

During glucose infusion the balances of glucose and pyruvic acid became increasingly positive.

During intravenous injection of sodium succinate the balances of pyruvic, citric, and malic acids became significantly negative. Glucose balance did not change. The urinary excretions of pyruvic, citric, and malic acids were increased strikingly. There was a diminution in the percentage of filtered pyruvic acid which was reabsorbed by the renal tubules and a decrease in the net amount of citric acid reabsorbed. Part of the increase in malic acid excretion appeared to be accounted for by renal tubular secretion.

If it is assumed that a positive renal balance of a substance signifies its utilization by the kidney and that a negative balance implies renal formation of the substance, the above results demonstrate that human and dog kidneys *in vivo* have metabolic activities in addition to the recognized excretory functions.

*The Hemodynamic Actions of Protoveratrine in Essential Hypertension.* Charles W. Crumpton, Carl K. Friedland, Peter T. Kuo, Truman G. Schnabel, Jr., Elwood L. Foltz, Robert G. Page, Jesus Alanis and Joseph H. Hafkenschiel, Philadelphia, Pa. (Introduced by Calvin F. Kay).

Protoveratrine injected intramuscularly (6 micrograms per kilogram—18 patients) and intravenously (2 micrograms per kilogram—10 patients) decreased blood pressure markedly and produced a bradycardia of from 40 to 60, over a two hour period. Pressures remained low for four hours after intramuscular injection. Unpleasant symptoms were less severe than with "purified" veratrum viride extracts, but there was pain at the injection site. Intravenous dosage lowered blood pressure 30% for at least one hour, with minimal unpleasant symptoms and much less bradycardia. Oral dosage of 0.75 to 1.125 mgm., in 8 patients, induced a significant hypotension in 4, with unpleasant symptoms and complete heart block in 3 of these patients. No significant change in the circulation was observed in the other 4, who received 0.75 mgm.

Ballistocardiographic studies in 3 normal subjects and 2 hypertensive patients, before and after the intravenous dosage, showed an increase in the size of the wave form suggesting an increase in stroke volume, along with hypotension and bradycardia. However, direct recording of central aortic pulse pressure waves under similar conditions did not indicate a significant increase in stroke volume.

Measurement of coronary hemodynamics and oxygen metabolism in anesthetized dogs (2.5 to 3.0 micrograms per kilogram intravenously) suggested a slight decrease in coronary vascular resistance at the time of the hypotension. However, the effects on circulation did not indicate that protoveratrine has a therapeutically desirable action under these conditions.

Cerebral circulation studies in seven hypertensive patients (2 micrograms per kilogram intravenously) showed a significant reduction in cerebral vascular resistance one hour after injection. Cerebral blood flow, cerebral arteriovenous oxygen difference, and cerebral oxygen consumption remained constant.

*The Metabolism of $I^{131}$ Labelled Proteins in Rabbits: The Sensitivity of the Antigen Elimination Rate as an Indi-*

*cator of Antibody Response.* GUSTAVE J. DAMMIN,* SAMUEL C. BUKANTZ,* FRANK J. DIXON and DAVID W. TALMAGE, Saint Louis, Mo.

Intravenously injected homologous globulin is eliminated from the blood in two distinct phases, a rapid dilution and a slow constant non-immune phase with a biologic half life (BHL) of 48 hours. The elimination of bovine gamma globulin (BGG) also presents initial dilution and non-immune phases, the latter continuing until the fourth day when a rapid elimination rate (BHL—16 hours) begins and continues until the 7–8th day when less than 0.1 per cent of the injected protein remains. Such tri-phasic elimination curves are obtained with total antigen doses of 1, 75 or 500 mgs. Circulating antibody appears inconstantly following the 1 mg. dose, but is detectable on the 7–8th day following larger doses, persisting until the 20th day. Following complete disappearance of antigen from the blood, there is no significant tissue radioactivity. About 80 per cent of the injected protein-bound $I^{131}$ can be recovered in the urine as non-protein-bound $I^{131}$, neither antigen nor protein-bound activity appearing in the urine.

Because (1) the rate of the third phase of antigen elimination in the normal rabbit roughly parallels elimination in the sensitized rabbit, (2) the appearance of circulating antibody coincides with the disappearance of circulating antigen, and (3) rapid antigen elimination can be induced with passive antibody, this phase has been identified as an immune phase.

Rabbits injected with 1 mg. of BGG may develop no circulating antibody, but on re-injection exhibit a specific anamnestic response. X-irradiated (250 R) rabbits injected with BGG may show a short rapid third phase and develop no circulating antibody but on re-injection will exhibit a specific anamnestic response. Thus, with minimal antigenic stimulation or partial suppression of the immune response, no circulating antibody appears but the presence of a rapid antigen elimination phase constitutes immediate evidence of an immune response.

*Adrenal Cortical Reserve in Severe Hyperthyroidism.* WILLIAM H. DAUGHADAY and A. LEWIS FARR, St. Louis, Mo. (Introduced by C. M. MacBryde).

Abnormal adrenocortical function in hyperthyroidism has been suspected by many investigators. We have studied seven patients with severe hyperthyroidism with basal metabolic rates between plus 50 and plus 100 per cent. In each patient eosinophil leukocytes, urinary 17-ketosteroids and urinary formaldehydogenic steroids were measured before and after ACTH. Twenty-five mg. of ACTH were given, then 10 mg. every six hours for 48 hours. The average excretion of formaldehydogenic steroids was 1.9 mg./day (normal 0.5–1.5 mg.) before ACTH and 1.9 mg. and 2.2 mg. on the first and second days of ACTH treatment. The average excretion of 17-ketosteroids was 12.0 mg./day before ACTH and 12.7 and 12.7 mg./day on the 1st and 2nd day of ACTH. Normal rise of 17-ketosteroids on 2nd day of ACTH is 6 mg./day. The average fall in eosinophils four hours after 25 mg. ACTH was 46 per cent. Four patients had less than 40 per cent drop in eosinophils. Normal fall is 76 per cent. Repeated studies of adrenal function were made in 4 other patients with severe hyperthyroidism.

*These results suggest: 1) that hyperthyroidism is a stimulus for increased adrenal activity and 2) that decreased adrenal reserve may be present in severe hyperthyroidism as measured by responses to ACTH of eosinophils, ketosteroids, and formaldehydogenic steroids.*

*Use of Mutants to Reveal a Vitamin (POB) Antagonizing the Chemotherapeutic Action of PABA.* BERNARD D. DAVIS,* New York, N. Y.

In contrast to the antagonism of PABA to sulfonamides, the mechanism of antirickettsial chemotherapy by PABA is not understood. This problem was unexpectedly solved during studies on biosynthetic paths in *Escherichia coli.* Certain mutants blocked at an early stage in aromatic synthesis, which grow slowly on a mixture of tyrosine, phenylalanine, tryptophan, and PABA, were found to grow more rapidly on shikimic acid, a precursor of these metabolites. Since growth on the quadruple supplement was accelerated by culture filtrate of the parent wild type strain, we postulated a fifth aromatic metabolite to be derived from shikimic acid. This filtrate factor was identified as p-hydroxybenzoic acid (POB); it is fully active at a concentration (0.01 $\mu$g/ml) characteristic of vitamins. Its sulfone analogue (4,4′-dihydroxydiphenyl sulfone) was found to be a competitive inhibitor of *E. coli.* On the basis of structural considerations PABA was similarly tested; it slowed growth of *E. coli* at 150 $\mu$g/ml, and was reversed by POB in a 1/200 ratio. Preliminary experiments of J. C. Snyder have shown that POB also antagonizes rickettsiostasis by PABA in animals and chick embryos. Since POB is not present in significant amounts in liver extract, and one analogue of it (PABA) is known to have chemotherapeutic utility, the expectation has been realized that mutants could reveal metabolites peculiar to microorganisms, and hence suitable as models for the synthesis of chemotherapeutic analogues.

*Measurement of Pulmonary Pressure.* HOWARD G. DAYMAN, Buffalo, N. Y. (Introduced by David K. Miller).

Pulmonary pressure, as previously reported, is the difference between lung tension and pleural pressure, all expressed as cm. HOH pressure. The difficulty is measurement of lung tension. Rate of airflow and pleural pressure are continuously recorded by apparatus of original design in patients having a small pneumothorax space. At the instant of apnea between breaths of varying size, lung tension and pleural pressure are identical, and it is shown that $\frac{\text{Lung Tension}}{\text{Lung Volume}} = \text{K}\ (+4\%)$. From the airflow tracing, change in lung volume and thereby change in lung tension may be computed at $\frac{1}{10}$th second intervals during active respiration. This method of computing lung tension, not previously described, is the only means by which trends in pulmonary pressure may be determined with any degree of accuracy. Knowing the rate of airflow, change in lung volume, pulmonary pressure, lung tension, and pleural pressure enables one to study the mechanical forces affecting ventilation. Resistance to airflow is of special interest (cm. HOH pressure necessary to produce 1000 cc/sec. airflow).

Limitations of method include: 1) Pulmonary pressure is certainly not uniform throughout the lung and the data thus express trends in pressure rather than absolute measurements. 2) Pneumothorax on one side disturbs pressure relations between the pleural cavities. Evidence is presented to indicate that such pressure difference is appreciable only in a position of extreme expiration. Tests were made, therefore, in the complemental air range so far as possible. 3) Computed resistance to airflow is inexplicably low at the inception (.1–.3 sec.) of both inspiration and expiration. Evidence indicates that this is due to sudden change in the size of dead space. 4) Lung tension/lung volume relationship is abnormal in advanced emphysema and lung tension can be computed only by plotting a curve expressing the relationship and applying that curve to a particular breath. The mechanics of ventilation in emphysema are so abnormal that such inaccuracy as may result from this method of computing lung tension is not serious.

*The Behavior of Human Thyroid Tumors Transplanted to the Anterior Chamber of Guinea Pig Eyes.* Brown M. Dobyns and Beatrice Lennon, Boston, Mass. (Introduced by Oliver Cope).

The survival and growth of human tumors after transplantation to the anterior chamber of the guinea pig's eye has been regarded as a means of studying the behavior of tumors and diagnosing malignancy. Malignant lesions are said to grow, while benign lesions are absorbed. Twenty-three thyroidal tumors (many malignant or questionably malignant) as well as control tissues have been transplanted into 228 eyes of guinea pigs. Many malignant tumors have survived and grown. Some tumors, regarded as morphologically benign, have survived in the eye for over one year contrary to expectation. The survival but failure of multiplication of these tumor cells raises interesting speculation.

Some very undifferentiated thyroidal tumors which originally had no resemblance to thyroid have, after habitation in the eye, differentiated and acquired a resemblance to normal thyroid.

During a period of four years, one very undifferentiated thyroidal tumor was retransplanted through nine generations and 444 eyes during which time it gave rise to almost every histological pattern of carcinoma found in the human thyroid. These changes may be related to the variable character of some carcinomas growing in man.

Illustrations of the histological pictures serve to document the changes that have been found.

*Acquired Hemolytic Anemia as the Presenting Syndrome of Lupus Erythematosus Disseminata.* Edmund L. Dubois, Los Angeles, Calif. (Introduced by Paul Starr).

The cause of the hematological abnormalities in disseminated lupus has been as great an enigma as the etiology of the disease; it is the purpose of this paper to present three patients with the previously unreported syndrome of acquired hemolytic anemia including positive Coombs test who subsequently developed the classic features of disseminated lupus. The second aim of the paper is to review the blood changes in this disease with the hope of showing that a causative mechanism is the secondary form of "hypersplenism" and to emphasize the importance of studies for Hargraves cells on all patients with evidence of any form of increased splenic activity.

The first and third patients had the classic pattern of acquired idiopathic hemolytic anemia. Soon after this they developed the other changes of disseminated lupus. As a result of these cases, routine "LE" studies were done on all patients with evidence of hypersplenism. In the course of this investigation, Case Two, who also presented herself with acquired hemolytic anemia, was diagnosed as lupus. Several days later bilateral pleural effusions appeared.

It is shown that there is no cross-reaction between the antibodies giving positive Coombs test and the LE factor.

The following changes may be due to increased splenic activity, namely, leukopenia, thrombocytopenia, and hemolytic anemia, provided that the bone marrow is hyperplastic. Leucopenia is well known in lupus and it is pointed out that the marrow is myelopoietically active.

Thrombocytopenic purpura has often been reported as an initial manifestation of disseminated lupus. Bone marrow studies show plentiful megakaryocytes not forming platelets.

The findings in these three patients and others suggest the hemolytic nature of the anemia in the early stages.

In view of the above it is postulated that hypersplenism of the secondary type plays a significant role in lupus.

*Changes in Connective Tissue Reaction Induced by Cortisone—in vivo Observations using the Rabbit Ear Chamber Technique.* R. H. Ebert,* Chicago, Ill.

Since earlier work in our laboratory using the rabbit ear chamber as an in vivo method of observation had provided insight into the dynamics of certain hypersensitive states, it was felt that a study of the effect of Cortisone on these reactions might provide basic information as to how this hormone modifies the inflammatory response. Tuberculous infection, the focal reaction, and serum sickness were chosen for study because considerable information about these reactions has been accumulated.

The inflammatory response was studied in 24 experiments using Cortisone (5–25 mg. daily) and 21 untreated controls. Cortisone produced certain consistent changes in the inflammatory response which were independent of the type of reaction studied.

1) In all reactions, vascular tone was better maintained in the Cortisone treated animal than in the untreated control. This effect seemed to be due primarily to a mainte-

nance of arteriolar tone. Even in the apparently normal chamber arteriolar tone frequently increased with Cortisone therapy and this effect was even more striking if non-specific inflammatory changes were present prior to treatment.

2) Cortisone appeared to maintain the integrity of vascular endothelium. In each of the reactions studied Cortisone treatment reduced the degree of damage to arteriolar and venule endothelium. Sticking of leukocytes to endothelium was diminished and swelling of endothelium was suppressed. Endothelium in treated animals tended to maintain its normal highly refractile appearance.

3) As a result of this increased integrity of endothelium there was a decrease in diapedesis of leukocytes and a reduction in exudate.

The modification of the inflammatory reaction by Cortisone in tuberculous infection, the focal reaction, and serum sickness was quantitative. If the stimulus was sufficiently intense there was no measurable reduction in the reaction.

### *Body Water, Water Distribution and Water Kinetics as Revealed by the Use of Deuterium Oxide.* I. S. Edelman and F. D. Moore,* Boston, Mass.

Following the rapid intravenous injection of $D_2O$, a characteristic arterial time-concentration curve is observed. Its early slopes are determined by cell permeability; its "equilibrium" value by total body water, and its late slope by water turnover ("biologic decay") rates. This paper deals with an analysis of these three phases of the heavy water curve. The pre-equilibrium portion of the arterial curve resolves into two rate components indicating two classes of rate—determining boundaries limiting the free diffusion of water in the living organism. In vitro dialysis of skeletal muscle containing $D_2O$, and arterial venous $D_2O$ curves in the isolated hind limb of the cat, support the concept of two types of cell areas (one exchanging water with its interstitial fluid environment rapidly and the other slowly), accounting for the two rates of distribution noted. The zero-time volume of dilution, obtained by extrapolation of the curve and the application of the isotope dilution principle, is a measure of the extra-cellular water. The mean values so obtained were 15 per cent of the body weight in humans and 17 per cent of the body weight in dogs. Using this tri-cameral system for water exchange and from the arterial curves, we calculated the internal rates of water exchange and the distribution of body water. In toto, the extracellular water exchanges with cell water at the rate of 21 per cent/min. in humans and 25 per cent/min. in dogs.

The derivation of the above data, and the normal human total body water throughout the life span will be described.

### ✓ *Excessive Losses of Sodium, Chloride, and Water After Administration of Cortisone or ACTH, Suggesting Transient Adrenocortical Insufficiency.* Leonard P. Eliel, Olof H. Pearson and Charles D. West, New York, N. Y. (Introduced by David A. Karnofsky).

In a series of patients with lymphoid tumors who had received cortisone or adrenocorticotrophic hormone (ACTH) for 3 or more weeks, marked asthenia, collapse, pyrexia, delirium, and death were observed in several instances after withdrawal of cortisone, while asthenia was usually noted after stopping ACTH. The sodium and chloride balances, and body sodium and water contents were determined in one of these patients, the balances alone in two others.

The first patient, after receiving ACTH (100 mg/day) for 30 days, lost 2.5 times more sodium and 5 times more chloride than were retained during hormone administration. An ensuing 36 day period of cortisone (100–200 mg/day) resulted in no significant salt retention, or loss on hormone withdrawal. Cortisone was subsequently given continuously for 7 months (64–171 mg/day), first intramuscularly and later orally. Hormone withdrawal then resulted on two occasions in extreme dehydration, pyrexia, delirium, a fall in serum sodium, chloride, and pH levels, and marked urinary losses of sodium and chloride. The body sodium content estimated by Na$^{24}$ dilution during hormone deprivation showed a reduction to 34.0 meq/Kg from a value of 51.7 meq/Kg before hormone administration, while the total body water simultaneously estimated by antipyrine dilution showed a reduction of 11 liters. Exhibition of oral cortisone was followed by prompt clinical improvement and partial repair of the sodium and chloride deficit.

Large transient losses of sodium and chloride exceeding previous retentions by factors of 2 to 4.5 have been observed in two other patients after stopping administration of ACTH or cortisone.

The symptoms and excessive losses of sodium, chloride, and water observed in these patients following withdrawal of cortisone or ACTH, suggest adrenal cortical insufficiency which is usually transient but potentially fatal.

### *Observations on the Mechanism of the Hyperlipemia in the Nephrotic Syndrome.* Kendall Emerson, Jr.,* Marcel Roche, Stanley S. Kahn, Hugo W. Moser and Dalton Jenkins, Boston, Mass.

In four adolescent and young adult patients with the nephrotic syndrome treated with ACTH for 7 to 14 days (80–100 mgs. daily in four divided doses) the following changes were observed. The initially high R.Q. fell transiently in the first 24 hours of treatment, then rose well above the initial level, frequently exceeding 1.00, reached a peak about the fifth day and tended to fall slowly thereafter even while treatment was continued. The serum level of phospholipids and either total or esterified cholesterol or both also rose during the first 5 days of ACTH treatment, falling slowly from then until withdrawal of ACTH and rapidly thereafter in conjunction with the diuresis which invariably occurred. The serum protein bound iodine rose from very low initial levels toward normal.

In a series of control patients similarly treated, including two normal subjects, two patients with hypothyroidism, one with Cushing's Syndrome and one with idiopathic edema the R.Q. invariably fell during ACTH administration; no consistent variation occurred in the serum lipid levels and, except in the patients with hypothyroidism, the serum protein bound iodine level decreased.

It is concluded that the metabolic orientation of the nephrotic patient is in the direction of the conservation of fat. To this end his circulating thyroid hormone is decreased and adrenal stimulation calls forth a paradoxical response, namely increased combustion of carbohydrate and increased storage or synthesis of fat. It is suggested that this altered response represents a profound metabolic adaptation on the part of the liver, possibly analogous to the phenomenon of hibernation. Further studies are in progress to determine by means of $P_{32}$ turnover rates whether the rise in phospholipids represents increased synthesis or decreased utilization.

### *The Hemostatic Defect in Thrombocytopenia as Studied by the Use of ACTH and Cortisone.* WILLIAM W. FALOON, RICHARD W. GREENE AND EUGENE L. LOZNER,* Syracuse, N. Y.

Improvement in hemostasis and vascular resistance despite persistence of thrombocytopenia may follow splenectomy for purpura. Surgical procedures other than splenectomy may result in transient hemostatic improvement. Since these observations suggested the presence of humoral factors following stress, the effects of ACTH and Cortisone on the hemostatic defect in thrombocytopenia were investigated in four patients; two non-splenectomized adolescent girls, one man, 58 years old, and one woman, 66 years old. The man had been splenectomized one year previously without remission in thrombocytopenia or vascular fragility. The woman had been splenectomized six years previously with temporary remission of thrombocytopenia and subsequent relapse. The hemostatic studies included observations on vascular fragility (tourniquet test), platelet counts and the coagulation phenomena (prothrombin utilization and clot retraction) associated with changes in platelets.

During the administration of ACTH to three patients, all showed prompt improvement in vascular fragility. In the two girls, platelet counts and associated coagulation findings subsequently became normal. No imrovement occurred in the thrombocytopenia of the older man. In all three patients, discontinuance of ACTH was followed within a relatively short time by a return of the hemostatic defects.

One of the ACTH-treated girls subsequently received Cortisone orally, with maintenance of normal vascular resistance, but with almost complete relapse in thrombocytopenia and associated abnormalities.

The 66 year old woman has received only Cortisone. Capillary resistance approached normal without change in the platelet count or coagulation studies.

The data suggest that different adrenal hormones may be responsible for separate improvement in the vascular resistance and the platelet counts of thrombocytopenic patients. These studies may explain the dissociation between vascular fragility and thrombocytopenia which is observed clinically.

### *Renal Hemodynamics and Salt and Water Excretion During Induced Congestion of the Inferior Vena Cava of Man.* SAUL J. FARBER, J. DEAVER ALEXANDER and LUDWIG W. EICHNA,* New York, N. Y.

Renal venous congestion has been postulated as a causative factor in the salt and water retention of congestive heart failure. In an attempt to evaluate the effect of venous congestion on renal hemodynamics and salt and water excretion in man, a method was developed whereby a balloon was inflated in the inferior vena cava, above and below the entrance of the renal veins. Inferior vena caval pressures of 160 mm. to 260 mm. saline were produced and maintained for 30 minutes. When the congestion included the renal veins, the excretion of water, sodium and chloride decreased markedly in 17 of 19 non-cardiac subjects. In the 7 subjects so studied, glomerular filtration rate and renal plasma flow decreased by approximately one-third during the first 20 minutes but began to recover during the last 10 minutes of congestion. In contrast, water and electrolyte excretions remained reduced or continued to fall throughout the period of congestion and returned to control values only after deflating the balloon. Neither the renal hemodynamic nor urinary changes were related to the degree of rise in venous pressure. Congestion of the inferior vena cava *below* the level of the renal veins induced rather similar effects; water and electrolyte excretion decreased in 6 of 9 subjects, and, in the 3 subjects studied, renal hemodynamics changed in the same manner as during inflation of the balloon above the renal veins.

Congestion of a sizable segment of the inferior vena caval drainage area appears to decrease the excretion of water and electrolytes and this reduction becomes practically invariable when renal congestion is present. Renal hemodynamic changes may contribute to the initial decrease in water and electrolyte excretion but are not necessary for the continuance of the effect. Factors other than renal venous congestion are involved in the antidiuretic effects observed.

### *The Role of the Pituitary Adrenocortical System in the Response to Anoxia.* THOMAS F. FRAWLEY, MARCEL ROCHE, DALTON JENKINS and GEORGE W. THORN,* Boston, Mass.

In the present studies an estimate of the amount of endogenously liberated ACTH during a single exposure to anoxia has been made and the influence of ACTH and cortisone upon the altitude tolerance of normal males has been investigated. Anoxia was produced by breathing through a closed spirometer system containing atmospheric air with all expired $CO_2$ being absorbed by soda lime. The experiment was terminated when the subject became unable to respond to light signals. A comparison between the fall in circulating eosinophils caused by anoxia and that produced by the injection of a known amount of ACTH furnished an approximate estimate of the amount of ACTH secreted spontaneously by the human pituitary in response to the stress of anoxia. The per cent change in circulating eosinophils 4 hours after acute anoxia ranged from 11 per cent to 62 per cent, with an average fall of 36 per cent. When compared to the fall obtained with ACTH in these same individuals, it appeared that under the circumstances of the test the anterior hypophysis secretes an amount of ACTH equivalent to slightly less than 25 mg. of injected ACTH.

ACTH in doses of 160 mg. daily over a five-day period produced an improvement in anoxia tolerance associated with an increased oxygen uptake. In five normal subjects the average initial altitude tolerance measured 28,800 feet, with a range of 23,200 to 32,700 feet. The average altitude tolerance during the administration of ACTH was 30,400 feet, with a range of 28,000 to 34,000 feet. Five hundred mg. of cortisone administered orally to two normal subjects over a 6-hour period preceding the anoxia test produced a change in altitude tolerance from 29,575 to 32,620 feet. Temporary pituitary-adrenocortical unresponsiveness to anoxia developed following ACTH and cortisone.

*Reduction of Blood Pressure in the Normotensive and Hypertensive Animal Following Potassium Deprivation.* Meyer Friedman,* S. Charles Freed and Ray H. Roseman, San Francisco, Calif.

Recent studies indicate that reduction of blood pressure occurs in rats following restriction of potassium in their diet (0.01 per cent of total diet). Thus the average blood pressure of 33 *normotensive* rats fell from a control level of 103 mm. of Hg (Range: 88 to 128) to 87 mm. of Hg (Range: 66 to 116) after 7 weeks of potassium deprivation. Control rats had an initial blood pressure of 106 mm. of Hg (Range: 94 to 122) which rose to 116 mm. of Hg (Range: 102 to 130).

Similar potassium restriction had a much greater depressor effect on *hypertensive* rats. Thus the average pressure of 13 hypertensive rats fell from 165 mm. of Hg (Range: 140 to 200) to 123 mm. of Hg (Range: 100 to 150) after 7 weeks of potassium deprivation. The blood pressure of the control hypertensive rats rose from 153 mm. of Hg (Range: 140 to 165) to 171 mm. of Hg (Range: 140 to 198) during the same period.

This depressor effect of potassium restriction could be prevented by concomitant restriction of sodium (0.04 per cent of total diet). However, excessive sodium intake (0.70 per cent of diet) did not accentuate the depressor effect of potassium restriction.

Although foci of necrosis could be found in the myocardium of potassium deficient rats, no correlation could be established between severity of lesion and degree of induced hypotension. However, the pressor response of the potassium deficient rat to injections of epinephrine, renin, angiotonin and norepinephrine were respectively ½, ⅔, ½, and ⅓ those of the normal rat. These findings suggest that the depressor effect results from a loss of *peripheral* vascular responsiveness.

*Metabolism of Pteroylglutamic Acid and Citrovorum Factor in Human Scurvy.* George J. Gabuzda, Jr., Gerald B. Phillips, Robert F. Schilling and Charles S. Davidson,* Boston, Mass.

The urinary excretion of citrovorum factor (CF) was determined microbiologically following pteroylglutamic acid (PGA) administered orally to two men with scurvy before and during ascorbic acid therapy.

The patients were fed scorbutic diets. During the first three days urinary CF was 0.3 gamma, and 1.0 gamma daily, respectively. When 10 mg. of PGA were administered daily for six days to one patient, CF excretion became 8 to 16 gamma daily. During the next six days 1 gm. of ascorbic acid was given intramuscularly daily to this patient in addition to PGA. Urinary CF was 15 gamma the first day of ascorbic acid therapy, 30 gamma the second day, and ranged from 95 to 126 gamma daily for four additional days.

The second patient's urinary CF ranged from 9 to 13 gamma daily when 10 mg. of PGA were given daily for three days. One gm. of ascorbic acid was then given orally daily for seven days, in addition to PGA. Urinary CF was 17 gamma the first day of ascorbic acid therapy and increased to 110 gamma by the seventh day. PGA was then omitted, but ascorbic acid continued. Urinary CF returned promptly to initial levels. After six days PGA was given again in addition to ascorbic acid. On the first day urinary CF rose to 94 gamma, and averaged 133 gamma daily during three additional days.

*In vitro* measurements indicate that the quantities of PGA and of ascorbic acid excreted by these patients do not invalidate the microbiological assay of CF.

Conclusion: A small amount of CF was excreted in the scorbutic state and was increased by orally administered PGA. However, maximal CF excretion occurred only when both PGA and ascorbic acid were given. Thus, ascorbic acid facilitated conversion of PGA to citrovorum factor in man.

*Ambient Air Recording Bronchospirometry and Differential Residual Volume Determination.* Edward A. Gaensler and David W. Cugell, Boston, Mass. (Introduced by Richard A. Bloomfield).

Conventional recording bronchospirometry requires the breathing of high oxygen mixtures. This places the diseased lung at a relative advantage and conclusions from its performance under these conditions do not necessarily apply to function under room air tensions. Differential lung volume determination is not possible because the initial concentrations of gases in the lungs are not known.

To obviate these difficulties a bronchospirometer fitted with twin air tight boxes and balloons was used which recorded respirations reflected by pressure changes within the boxes. One or both lungs could be supplied with ambient air or oxygen and the inspiratory gas composition could be changed instantaneously.

In normal volunteers oxygen and ambient air bronchospirometry resulted in the same relative oxygen uptake and ventilatory equivalent. Partially destroyed lungs showed up to twice the relative oxygen uptake with high oxygen tensions than with ambient air. The functional effectiveness of diseased lungs determined by high oxygen bronchospirometry is therefore overestimated.

Ambient air bronchospirometry was applied to the open circuit residual volume method of Darling, Cournand, and Richards. It solved one of the problems of this method by permitting visualization of the exact place in the respiratory cycle at which both lungs were turned into the oxygen circuit. The true functional residual volume was calculated after correction from the spirogram of the preparatory ambient air breathing period.

In normal volunteers the functional residual volume distribution corresponded closely to distribution of oxygen uptake, ventilation, and vital capacity. In a group of 40 patients with partially destroyed lungs the relative functional residual volume was often many times greater than other indices of function. This discrepancy was greatest in "imprisoned lungs" of fibrothorax and chronic empyema. Pulmonary mixing indices, often normal after combined oxygen breathing, showed marked mixing impairment by differential alveolar air sampling.

*Observations on Insulin Labeled with Radio Iodine.* JOSEPH W. GARDELLA and JOSEPH W. FERREBEE,* Cooperstown, N. Y.

Bovine insulin several times recrystallized from phosphate buffer has been labeled with radio iodine in amounts of 10 to 1000 microcuries of $I^{131}$ per milligram of insulin, the total quantity of $I^{127}$ and $I^{131}$ organically bound being of the order of two atoms per molecule of insulin. Studies of insulin so labeled have revealed ranges within which the labeled insulin appears to have unimpaired biologic activity as measured by mouse convulsion test or by the acceleration of glycogen synthesis in isolated rat diaphragms. Physical chemical studies reveal no changes in salt precipitability of labeled insulin as compared with native insulin and no changes in adsorption on cellulose, indicating that the label has not greatly distorted the molecule. From these in vitro and in vivo observations it has been concluded that iodine labeled insulin may be used within limits to study the mechanism of insulin action and insulin metabolism. The pattern of localization of radioactivity in various organs following the injection of labeled insulin will be presented and the effect of previous administration of native insulin, glucose and other factors affecting carbohydrate metabolism will be reported. If time permits and appropriate AEC permission is granted, blood and urine curves of radioactivity following the injection of radio insulin into normal, diabetic and insulin-resistant individuals will be presented.

*Cardiac Output in Acute Myocardial Infarction.* ROBERT P. GILBERT, STEPHEN L. ALDRICH and LYNN ANDERSON, Chicago, Ill. (Introduced by Richard B. Capps).

The dye dilution technique for determining cardiac output, as described by Hamilton et al and as modified by Ebert et al, for dye collection offers a method which can be used at the bedside in sick patients.

So far three cases of acute myocardial infarction have been successfully studied by this technique. Arterial pressures have been recorded by a strain gauge manometer and venous pressures and circulation times determined by conventional methods. Two patients had low outputs and somewhat elevated pressures. One patient who had just recovered from a period of very low pressure was found to have a high output which suggests that his previous drop in pressure may have been associated more with a decreased total peripheral resistance. Particular attention will be paid to patients with low pressures in an effort to determine the relative importance of decreases in cardiac output and in total peripheral resistance.

*Mechanism of Production of Lung Lesions in Virus Pneumonia.* H. S. GINSBERG and F. L. HORSFALL, JR.,* New York, N. Y.

Lung lesions developing during experimental virus pneumonia induced with influenza virus or pneumonia virus of mice (PVM) increase at a rate much slower than the rate of increase in virus concentration. Moreover, high concentrations of Newcastle disease virus (NDV) cause extensive lung lesions in the mouse but no multiplication of this virus occurs. The gross and microscopic pathology of lung lesions caused by influenza, PVM, or NDV are indistinguishable. These findings indicate that in virus pneumonia factors other than the process of virus multiplication damage host cells.

The lung lesion factor is an integral component of the infective NDV particle itself: It is absorbed by and eluted from erythrocytes along with the active virus; it is not separable from the virus in high gravitational fields; and it is inactivated by either heat or ultraviolet irradiation at a rate closely similar to that of the infective virus.

Lung lesions caused by high concentrations of NDV develop at a rate similar to that found with influenza virus. Production of pneumonia by NDV is formally analogous to a virus infection: lung cell-virus combination is prevented by treatment of the living lung with receptor destroying enzyme (RDE), and in such treated lungs NDV does not cause lesions; NDV immune serum specifically prevents lung lesion production when given before or with virus but has no effect when given 30 minutes after; and prior multiplication of unadapted influenza virus in the lung completely inhibits lung lesion production with NDV.

These results support the following postulate: When a sufficiently high concentration of infectious virus is attained, either by multiplication or direct inoculation, cellular damage is caused. Lung lesions in virus pneumonia then are in large part attributable to the cytotoxic effects of the virus particle *per se*.

✓*The Effect of Cortisone on Acute Bacterial Infections.* ROBERT J. GLASER, JOHN W. BERRY, LENORE H. LOEB and W. BARRY WOOD, JR.,* St. Louis, Mo.

Cortisone and ACTH are known to depress a variety of inflammatory states, both in patients and experimental animals. Dramatic defervescence and subsidence of symptoms have resulted from their use in pneumococcal pneumonia, in spite of bacteriological evidence of continued infection. The experiments here reported have been designed to study the effect of Cortisone upon the course of acute bacterial infections and particularly upon the cellular response of the host.

Streptococcal and pneumococcal pneumonia were produced in rats by intrabronchial inoculation. Half of the animals were treated with Cortisone beginning five days before infection, while the other half served as untreated controls. The mortality rate for each group was determined, and a systematic histologic study was made of the infected lungs. Similar studies were made in mice with streptococcal cervical adenitis produced by intranasal inoculation.

The survival rates in all experiments were less favorable among the animals treated with Cortisone. The host response to the infectious stimulus was strikingly altered. The lesions in the Cortisone treated animals contained excessive amounts of relatively acellular edema fluid in which there were many more bacteria than were ever seen in analogous lesions from control animals. The sparsity of cells in the edema fluid and the extraordinarily large bacterial population appeared to be related to a delay in the diapedesis of leucocytes. Cortisone *per se* was shown to exert no growth-stimulating effect upon the bacteria, nor did it influence significantly the phagocytic activities of the leucocytes which eventually appeared in the lesion.

Further studies are in progress relating to the mechanism whereby Cortisone depresses the process of cellular exudation, which in the case of acute bacterial infections constitutes one of the principal defenses of the host.

*Dynamics of Eisenmenger's Complex.* H. GOLDBERG, E. N. SILBER and A. GORDON, Chicago, Ill. (Introduced by L. N. Katz).

The patho-physiology underlying many of the manifestations of Eisenmenger's Complex has remained obscure. Chief among these is the delayed onset of cyanosis which is claimed to result from some congenital abnormality of the pulmonary epithelium or capillaries. A case of Eisenmenger's complex studied by cardiac catheterization is presented in which, in addition to the usual features, anomalous pulmonary venous drainage into the right auricle was demonstrated by entrance of the catheter into one of these veins. This afforded the opportunity to measure directly the total vascular resistance to blood flowing through the lungs and to determine whether or not a pulmonary factor for the production of cyanosis exists in this condition.

The pulmonary venous pressure and pulmonary blood flow were normal. It is concluded that the elevated pulmonary arterial pressure is due to an increased resistance resulting from a narrowing of the peripheral pulmonary vascular bed. This narrowing may be due to functional as well as organic changes. Although hypoxemia plays a role, it cannot account for all of the increased vascular resistance. Organic changes affecting the medial and intimal layers of the muscular arteries of the lung play a major role. The intimal changes are progressive and appear to be secondary to the pulmonary hypertension.

Blood obtained from the pulmonary vein was fully saturated. This indicates that the peripheral unsaturation is due solely to veno-arterial shunting of blood. This eliminates the presence of a pulmonary factor as a cause for the cyanosis in Eisenmenger's Complex.

The principles of surgical correction of this disease are discussed and may be summarized as follows:

1. A reduction of the pulmonary artery pressure.
2. Alleviation of the strain upon the right ventricle.
3. Return of the peripheral arterial saturation toward normal. Correction of the overriding aorta with elimination of the ventricular septal defect would accomplish these objectives.

*Nor-epinephrine and Epinephrine in Human Urine (Addison's Disease, Essential Hypertension, Pheochromocytoma).* MARCEL GOLDENBERG and MAURICE M. RAPPORT, New York, N. Y. (Introduced by Robert F. Loeb).

The inactivation mechanisms and the metabolic fate of epinephrine and nor-epinephrine are not established. Ingested epinephrine is partly excreted in the urine as a sulfate ester (Richter). Normal human urine contains a mixture of catechol-amines (epinephrine, nor-epinephrine and perhaps hydroxytyramine) (Holtz). This mixture is roughly composed of 85–90 per cent nor-epinephrine and approximates the composition of the sympathetic transmitter rather than that of the adrenal medullary hormone (70–90 per cent epinephrine). The normal daily excretion ranges from 15–45 micrograms nor-epinephrine in 24 hours (von Euler).

In an attempt to establish the source of these urinary catechol-amines, 7 cases of Addison's disease maintained on salt, Doca and cortisone were investigated. Of these, 5 were due to atrophy, in which case the adrenal medulla may have been preserved. Four of these showed a normal excretion, one a sub-normal value. Two cases of tuberculosis of the adrenal glands showing extensive calcification of both adrenals (in which it can be safely assumed that there is no remaining functioning medulla), showed an excretion of nor-epinephrine which was high normal in one case and far above normal in the other (maintained on cortisone). It therefore can be assumed that the adrenal medulla is not the principal source of the catechol-amines excreted in the urine. Since extra-adrenal chromaffin tissue is known to regress completely after fetal life except for small cell clusters in the sympathetic ganglia, the source must rather be the sympathetic or some other tissue. Three cases of extensive lumbo-dorsal sympathectomy showed the lowest observed sub-normal values. These findings are compatible with Cannon's "emergency" theory of adrenal medullary function which states that the resting secretion of the adrenal medulla is negligible.

The recent claim (Holtz) that an excessive excretion of nor-epinephrine occurs consistently in essential hypertension was not confirmed. Out of 14 cases only 2 showed an excretion above normal.

Finally, 2 cases of pheochromocytoma were investigated and found to excrete quantities of epinephrine and nor-epinephrine far in excess of the values obtained in normals and hypertensives (confirming Engel and von Euler).

In one of these cases the daily excretion amounted to 1600 micrograms of epinephrine and 400 micrograms of nor-epinephrine. Both cases showed the clinical characteristics of pheochromocytoma with persistent hypertension and repeatedly gave positive benzodioxane tests. Whether the urinary excretion is increased in the "non-humoral" phase of persistent hypertension due to pheochromocytoma, which poses the major diagnostic problem, is under investigation.

### *Studies in Diurnal Variation of Water and Electrolytes: Nocturnal Diuresis of Water and Sodium in Congestive Cardiac Failure, Cirrhosis of the Liver and Degenerative Glomerulonephritis.* RALPH GOLDMAN, Los Angeles, Calif. (Introduced by Samuel H. Bassett).

The mechanism of sodium retention in edema-forming diseases has been the subject of much study and controversy. Two concepts dominate. The first theory is that sodium is reabsorbed at a fixed rate from the filtrate, and that the amount of sodium in the urine is related to the rate of glomerular filtration. The other theory is that the filtered sodium is subject to variable tubular reabsorption. Data obtained by others and ourselves indicate that in congestive cardiac failure there is often a nocturnal diuresis of sodium as well as water. This has been explained by a nocturnal increase in the glomerular filtration rate resulting from a decreased peripheral demand for blood during the resting state. Examination of the diurnal excretory cycle in thirteen patients with cirrhosis of the liver and ascites revealed that ten had a clear-cut nocturnal diuresis of water and sodium. A similar study of five patients with degenerative glomerulonephritis revealed that two of these patients also demonstrated a nocturnal sodium diuresis. The potassium excretion in two-thirds of these patients showed a daytime maximum, although the curves were moderately flattened. While it is possible to conceive of renal hemodynamics as the chief factor influencing nocturnal diuresis in congestive cardiac failure, there is no data to suggest that the nocturnal diuresis seen in cirrhosis of the liver and glomerulonephritis has the same cause. With the available data, it appears more probable that in these conditions, and perhaps in cardiac failure as well, the regulation of sodium reabsorption depends upon variations in tubular metabolism controlled by a humoral agent which can be affected by the functional state of the liver. Data will be presented.

### *The Effect of Mild Diabetes and Insulin on Heart Muscle Metabolism in Man.* WALTER T. GOODALE, ROBERT E. OLSON and DONALD B. HACKEL, Boston, Mass. (Introduced by James P. O'Hare).

Studies on normal individuals by coronary venous catheterization have shown consistent patterns of extraction, and presumed utilization, of glucose, lactate and pyruvate by the myocardium. The coronary arteriovenous difference of each metabolite was related directly and linearly to its own arterial level, in both man and dogs. No significant myocardial glucose utilization occurred, however, below a mean arterial glucose level of $60 \pm 10$ mg. per cent, while lactate and pyruvate utilization was detectable down to arterial levels of 2.5 and 0.5 respectively. At average non-fasting arterial glucose, lactate and pyruvate levels of 103, 7.5 and 1.3, average coronary arteriovenous differences were 10.7, 2.7 and 0.4, and the myocardial RQ (MRQ), 0.86 – 0.93. At *low* fasting arterial levels, glucose, lactate and pyruvate extractions were correspondingly small or negligible, and MRQ near 0.7.

Five patients with minimal asymptomatic diabetes showed negligible myocardial glucose extraction despite fasting glucose levels of 95 – 216, with greatly reduced lactate and pyruvate utilization and an MRQ of 0.67 – 0.71. Insulin restored lactate and pyruvate utilization to normal, and initially raised the diabetic MRQ to 1.1 – 1.2, accompanied by very high coronary A–V glucose differences up to 29 mg. per cent as the arterial glucose level began to fall. Far more glucose was extracted than was needed theoretically as substrate for the simultaneous oxygen extraction, suggesting a partially anabolic non-oxidative utilization of glucose under the influence of insulin. With further fall in glucose level toward normal following insulin, the glucose, lactate and pyruvate utilizations and MRQ approached values seen in normal non-fasting individuals. These results suggest that insulin may promote phosphorylation of not only glucose but also other substrates such as pyruvate, and demonstrate an abnormal pattern of heart muscle metabolism in even minimal diabetes.

### *The Effects of Quiet Standing on Soluble Diuresis.* A. V. N. GOODYER and D. W. SELDIN, New Haven, Conn. (Introduced by F. G. Blake).

The sharply decreased excretion of sodium during quiet standing is due at least in part to a potent stimulus for the increased tubular reabsorption of sodium and is usually not prevented by the administration of hypertonic saline.

It was of interest to investigate the extent to which this stimulus might interfere with the augmented excretion of sodium promoted by the administration of mannitol, or of sodium salts of acids, the anions of which must be rapidly excreted.

The experiments were performed in normal adults, most of whom had been deprived of water for 12 hours or more. After several preliminary periods in the supine position, each subject stood up and, 10–15 minutes later, an isotonic or hypertonic solution of mannitol, sodium bicarbonate or sodium phosphate was administered.

It was found that quiet standing limited greatly the usual augmentation of sodium excretion caused by mannitol diuresis. In several cases, the excretion of sodium actually decreased in the face of a concomitant mannitol diuresis. However, the usual relationship between total solute excretion and urine flow during water restriction was disturbed on only one occasion.

Quiet standing did not limit the usual increase in sodium excretion produced by the administration of sodium phosphate or sodium bicarbonate.

Measurements of GFR and RPF in some experiments indicated that the changes in sodium excretion were primarily of tubular origin.

It is concluded that: 1) the rate of sodium excretion in mannitol diuresis is not strictly related to the quantity of mannitol in the tubular urine; it may be conditioned greatly by internal environmental factors affecting the renal tubular reabsorption of sodium; 2) the tubular rejection of sodium which attends the excretion of bicarbonate and phosphate takes precedence over the usual retention of sodium produced by quiet standing.

### *Circulatory Changes in Mitral Stenosis Following Valvuloplasty.* Richard Gorlin, Lewis Dexter,* and Dwight E. Harken, Boston, Mass.

Eleven patients with mitral stenosis were studied before and 2 to 6 weeks after mitral valvuloplasty by the technique of cardiac catheterization. Cardiac outputs were measured by the direct Fick method. Pressures were recorded in the pulmonary "capillaries," pulmonary artery, and right heart with electromanometers. Mitral valve orifice areas were calculated from a hydraulic formula described previously. Mitral regurgitation was detected from the pulmonary "capillary" pressure pulse. Pulmonary resistances were calculated from standard formulae. The mitral valve orifice was significantly widened in 10 of 11 patients. In only one instance was mitral regurgitation produced at operation. In three others with regurgitation preoperatively, no increase occurred with valvuloplasty. Pulmonary "capillary" pressure decreased in 9, while cardiac output remained essentially unchanged in 9 and increased in 2. Pulmonary arteriolar resistance decreased in 5 of the 9 patients in whom it was elevated. Right ventricular diastolic filling pressure fell towards normal in 4 of 6 patients in whom it was elevated. Total pulmonary resistance, a measure of right ventricular load, decreased in 8. It is concluded that valvuloplasty effectively widened the mitral stenotic orifice without the production of regurgitation. Although the widened area was only 20–50 per cent of the normal of 4–6 cm.$^2$, this was sufficient to produce definite hemodynamic improvement because the reserve capacity of the mitral valve is approximately 80 per cent. If stenosis was severe, prior mitral regurgitation did not militate against successful valvuloplasty. Following operation, pulmonary congestion and pulmonary arteriolar resistance tended to decrease and right ventricular function to improve. These physiologic alterations have been attended by dramatic improvement in the clinical state of most of these patients. Long-term follow-up is desirable, although few such studies have been carried out to date.

### *The Specificity of the Relation Between Attitudes and Diseases.* William J. Grace and David T. Graham, New York, N. Y. (Introduced by David P. Barr).

One hundred twenty-seven patients with one or more of 12 different bodily disturbances were interviewed to determine whether a specific attitude was associated with each symptom. When the life situation to which the patient was reacting had been clearly defined, he was asked to give a clear and ambiguous statement as to, a) how he saw himself in the situation, and b) what he wished to do about it at the time of the occurrence of the symptoms. These two components define the *attitude.* Conventional names of emotions, e.g. "anger" were not accepted. Only direct statements of patients, without reference to dream or associative material, were used in the study.

It was found that there was a specific attitude associated with each of the folowing symptoms: 1) Urticaria occurred in patients who felt they were being mistreated and were helpless. 2) Eczema occurred when the individual felt he was being interfered with or prevented from doing something and could do nothing about it. 3) Cold moist hands were noted in individuals who wanted to undertake some kind of activity. 4) Vasomotor rhinitis and asthma occurred in those who did not want to have anything to do with their problems. 5) Diarrhea occurred in persons who were wishing to get rid of their problems. 6) Constipation occurred in those who were grimly determined to go on in the face of difficulties. 7) Nausea and vomiting occurred when an individual was thinking of something which he wished had never happened. 8) Duodenal ulcer occurred in those who were seeking revenge. 9) Migraine headache occurred in an individual after he had been making an intense effort. 10) Arterial hypertension occurred in individuals who felt that they had to be prepared to meet all possible threats. 11) Low back pain occurred in those who wished to carry out some action involving the whole body, such as running away.

Many of these symptoms seem to be biologically appropriate to the attitude they accompany.

### *Evidence for an Adrenal Phase of Gastric Secretion.* Seymour J. Gray, Robert W. Reifenstein, Howard M. Spiro and John A. Benson, Jr., Boston, Mass. (Introduced by Marshall N. Fulton).

ACTH administered to experimental subjects in doses of 80 to 120 mgm. daily for 10–21 days produced an immediate and marked increase in the 24-hour urinary excretion of uropepsin and a significant rise in gastric juice pepsin within 7–14 days. A concomitant increase in gastric acidity was also observed on fasting serial aspirations and in the 12-hour nocturnal secretion. Both the concentration and total amount of hydrochloric acid, pepsin, and uropepsin were increased. An identical rise in uropepsin excretion was observed following the administration of cortisone orally or intramuscularly.

The administration of ACTH to a patient with a gastric ulcer resulted in a severe exacerbation of epigastric pain associated with marked epigastric tenderness, definite evidence of peritoneal irritation, localized protective muscle spasm, and guaiac-positive stools. The 24-hour excretion of uropepsin increased from 4900 to 9500 units during the 10 days of ACTH administration and was accompanied by a significant rise in gastric pepsin and acid secretion.

The increase in uropepsin, gastric pepsin, and gastric acidity (expressed as total mgm. of free HCl) approximated 100 to 400 per cent following ACTH administration.

A similar rise in the uropepsin excretion was observed following repeated injections of 0.6 mgm. epinephrine at 6-hour intervals for 4 days. This was associated with a significant fall in the level of circulating eosinophiles indicating a definite uropepsin response to adrenal stimulation.

The relation of this adrenal phase of gastric secretion to stress and the pathogenesis of peptic ulcer will be discussed.

### √ *Studies of Prevention of Some of the Toxic Effects of Prolonged Cortisone and ACTH Therapy.* L. GREENMAN, T. S. DANOWSKI,* R. TARAIL, J. H. PETERS, F. WEIGAND and F. MATEER, Pittsburgh, Pa.

Electrolyte and nitrogen excretion in stools during a whole milk regimen administered to hospitalized patients has been compared with the output during a sodium-free milk diet. Carbohydrate and electrolyte-free milk protein were added to provide adequate calories and nitrogen. On the markedly restricted intake of sodium a) the daily excretion of potassium, b) the potassium in 100 grams of formed stool and c) the stool output of potassium in relation to nitrogen all fell significantly ("p" for the "t" test was less than 0.05 upon comparison of the above findings with those observed during maintenance on a whole milk formula). The sodium and the nitrogen output were not detectably altered while a statistically significant, though from the point of view of magnitude small, drop in chloride output was observed in the per 100 grams of stool and per gram of nitrogen categories. The presence of larger amounts of potassium in the low sodium milk together with the more complete absorption of potassium from this source provides the organism with 20 milliequivalents of potassium per day in excess of that obtainable from a comparable whole milk formula. The attributes of such low sodium milk formulae have been employed for the complete prevention of edema, maintenance of positive balances of potassium and of nitrogen, and deferral, minimization or prevention of hypokalemia, hypochloremia and alkalosis in a large series of patients receiving cortisone or ACTH for as long as 68 days.

### *The Results of Feeding Desiccated Thyroid to Thyrotoxic Subjects.* MONTE A. GREER, Boston, Mass. (Introduced by Raymond D. Adams).

It has been observed that exogenous thyroid hormone in amounts of one to three grains daily will markedly suppress endogenous thyroid activity of normal human subjects, as measured by the accumulation of $I^{131}$. The BMR and PBI (serum protein-bound iodine) remain constant during such procedures and it has been assumed that this decrease in thyroid function has been brought about through suppression of pituitary thyrotrophin secretion.

It was therefore considered of interest to determine the effect of feeding thyroid hormone to thyrotoxic subjects. Following control determinations in sixteen untreated thyrotoxic patients, daily doses of desiccated thyroid were given, gradually increasing to twelve grains daily, increments being made at approximately weekly intervals. Thorough clinical evaluation, including pulse, weight, and $I^{131}$ uptake, was made at each visit in every patient. In suitable patients the BMR and serum protein-bound iodine were also determined. It was found that a gradual suppression of the accumulation of $I^{131}$ by the thyroid was produced, but that in no instance was this complete, never being less than 18 per cent/24 hours even when the patient was taking twelve grains of thyroid daily. The pulse, weight, BMR, and PBI, however, remained constant during the entire period of observation.

In one patient the 24-hour uptake of $I^{131}$ was reduced from 84 per cent to 33 per cent by the ingestion of nine grains of thyroid daily. Six weeks following the cessation of therapy the $I^{131}$ uptake was still at this low level and the patient was found to be accumulating $I^{127}$ in the thyroid at a rate equivalent to only 0.4 grains of thyroid per day.

Since the $I^{131}$ uptakes of 48 euthyroid subjects and 18 with simple goiter were all found to be suppressed below 15 per cent/24 hours by the administration of twelve grains or less of thyroid daily, it is suggested that the administration of twelve grains of thyroid daily for a period of two weeks may help to differentiate equivocal cases of hyperthyroidism.

### √ *Lack of Analgesic Effect of Cortisone and ACTH.* ALBERT W. GROKOEST, DEGUISE VAILLANCOURT, ROBERT GOTTSEGEN and CHARLES RAGAN,* New York. N. Y.

It has been suggested that the beneficial effects of cortisone and ACTH on diseases such as rheumatoid arthritis are in part due to an analgesic effect of these hormones. A systematic evaluation of cutaneous, dental and visceral pain has been undertaken in patients with rheumatoid arthritis, before and during the administration of cortisone and ACTH. Cutaneous pain was tested by intradermal injections of saline and distilled water; tooth sensitivity by electrical stimulation and visceral pain by distention of a balloon placed in the duodenum. The pain threshold and intensity were similar before and during the administration of the hormones and no true analgesia could be detected. It is proposed that the beneficial effects of hormone administration in rheumatoid arthritis and similar diseases evolve from a suppression of the inflammatory reaction in the host and not from a central or peripheral "analgesic" effect which cannot be demonstrated.

### *A Study of the Hemodynamic and Pathological Changes Induced by Bilateral Nephrectomy and Ligation of the Ureters of Dogs Maintained for Prolonged Periods by Means of Intermittent Peritoneal Lavage.* ARTHUR GROLLMAN, LOUIS B. TURNER and E. E. MUIRHEAD, Dallas, Texas (Introduced by Carl A. Moyer).

The elucidation of the role of the kidney, apart from its excretory function, in the animal economy has hitherto been rendered difficult because of the impossibility of maintaining the nephrectomized animal alive for extended periods. A procedure for intermittent peritoneal lavage has been devised which has permitted maintaining dogs alive in good condition for periods of months following bilateral nephrectomy, ligation of the ureters, or implantation of the ureters into the gut or vena cava. Following nephrectomy, the blood pressure gradually reaches hypertensive levels in the course of 1 to 2 weeks and remains so during the remaining life of the animal. The cardiac output, blood volume and venous pressure remain essentially unaltered, but there is an increase in peripheral resistance similar to that observed in hypertensive disease. Examination of the tissues of such animals reveals the acute lesions similar to those observed in the human dying of malignant hypertension. In animals maintained for a month or longer, the chronic lesions characteristic of those observed in the arterioles, myocardium and smooth muscle of the so-called benign form of hypertension in the human are observed. Animals retaining intact renal tissue (as when the ureters are ligated) and treated similarly develop neither hypertension nor these lesions. The results of these studies have an obvious bearing on the pathogenesis of hypertensive cardiovascular disease and indicate the role of the kidney in the maintenance of the normotensive state.

The method of intermittent peritoneal lavage offers a means of investigating other problems associated with renal function. It has been possible, for example, to differentiate the role of azotemia, the rise in blood pressure, etc. in the causation of the acute arteriolar necrosis observed in so-called malignant hypertension. The method as elaborated by the present study has also been applied successfully to the human.

*Determination of Cardiac Output in Man by the Fick Principle without Gas Analysis.* J. Grossman and R. E. Weston, New York, N. Y. (Introduced by Louis Leiter).

The usual method for measuring cardiac output by the Fick principle requires blood and air gas analyses with special laboratory equipment and procedures. However, cardiac output could be determined by cardiac catheterization without gas analysis by using any substance which is either added to or removed from the blood at an accurately measurable rate, if it were possible to produce an adequate difference between arterial and mixed venous blood concentrations of that substance. Unfortunately, the mixed venous (right ventricular) and arterial concentrations of substances added to or removed from only the greater circulation are identical. With a double lumen catheter, however, a measurable arterial-mixed venous concentration difference can be produced by injecting the test substance through the distal lumen, placed in the pulmonary artery. Mixed venous blood samples, then, can be obtained from the proximal lumen which opens into the right ventricle.

A number of potential test substances may be employed in this method. In these preliminary studies, sodium para-aminohippurate (PAH) was selected because of its virtually complete renal extraction, the large renal blood flow, the consequent high arterio-venous difference, and the ease of chemical determination of PAH in blood and urine.

It will be demonstrated that cardiac output may be calculated either as

$$\text{C.O.} = \frac{\dfrac{UV_{PAH}}{A_{PAH}V_{PAH}}}{1 - \text{Hct.}} \quad \text{or} \quad \text{C.O.} = \frac{\dfrac{C_{PAH}}{1 - \dfrac{V_{PAH}}{A_{PAH}}}}{1 - \text{Hct.}}$$

where $A_{PAH}$ and $V_{PAH}$ are arterial and mixed venous plasma concentrations of PAH, respectively, $UV_{PAH}$ and $C_{PAH}$ are the urinary excretion rate and renal clearance of PAH, respectively, and Hct. is the hematocrit.

Data on cardiac outputs concurrently measured in patients by the oxygen and PAH methods will be presented and discussed.

*Central Nervous System Sequelae as Shown by Post-Recovery Electroencephalograms in Rickettsial Spotted Fever.* George T. Harrell,* Richard L. Masland and Marvin J. Rosenblum, Winston-Salem, N. C.

Symptoms of encephalitis have been observed for many years in patients with rickettsial diseases. In patients dying during the acute phase of the disease pathologic sections often show either the acute microinfarct with arterial thrombosis and necrosis or the more chronic granulomatous "typhus nodule." Little information has been previously available on the residual damage to the central nervous system resulting from these infections.

Fifty-eight patients with rickettsial infections have been successfully treated in our clinic. Forty-three of these patients have been followed for periods varying from two months to eight years after the acute infection. At the time of re-evaluation a neurological examination and an electroencephalogram have been performed. The age of the patient and the type of treatment, whether supportive or specific, has been correlated with residual objective changes.

Even though these patients may be asymptomatic at the time of follow-up, abnormal electrical activity of focal, generalized or borderline character may be detected in electroencephalograms. Neurological findings are variable; in most patients the findings are normal, but in others bodily asymmetry, reflex changes, often involving the pyramidal system, or impairment of fine motion are noted. Four children have developed a convulsive disorder. The remaining children may be more easily disturbed emotionally, even though the electroencephalograms are normal. Some of the adults complain of impaired general health without specific localization of symptoms.

Many other types of infection may be accompanied by minor neurologic abnormalities during the acute febrile phase. Other disease processes, whether or not accompanied by high fever, show vascular changes pathologically which resemble in some aspects those of rickettsial diseases. Diffuse brain damage as a sequela of acute infection in diseases where the etiologic agent does not specifically parasitize nerve cells may be a more general phenomenon than is now recognized.

### *Demonstration of a Thrombocytopenic Factor in the Blood of Patients with Idiopathic Thrombocytopenic Purpura.* William J. Harrington, J. William Hollingsworth, Virginia Minnich and Carl V. Moore,* St. Louis, Mo.

The existence of a circulating thrombocytopenic factor has been demonstrated in six patients with idiopathic thrombocytopenic purpura. Three had developed thrombocytopenia acutely, one had chronic thrombocytopenia for at least three years and in two the disease had recurred after splenectomy.

The thrombocytopenic factor was demonstrated by transfusing 250 to 500 cc. of citrated whole blood or 100–300 cc. of citrated plasma from these patients into normal subjects or into patients with incurable disease. When the larger amounts of whole blood or plasma were given intravenously, the platelet level fell in the recipients within 30 minutes and frequently was less than 10,000 per cu. mm. at the end of one to two hours. In the more severe reactions purpura resulted. Thrombocytopenia persisted for four to six days, during which time megakaryocytes showed characteristics of immaturity.

ACTH, given to one recipient, did not prevent the effects from occurring. That the spleen is not required for the action of the thrombocytopenic factor, was demonstrated by the fall which occurred in one recipient whose spleen had been removed at the time of gastric resection for carcinoma of the stomach. However, blood obtained from one of the patients both three and ten days after splenectomy, at which times her platelet counts were 1,000,000 per cu. mm., caused a moderate platelet decrease in a normal subject.

The thrombocytopenic factor was stable for at least eight to ten days at −5°, 5°, and 26°C. It has been found in the plasma of all patients with idiopathic thrombocytopenic purpura studied to date and has not been demonstrated in the blood of control subjects.

Attempts are currently being made to determine whether platelet lysis or agglutination is responsible for the platelet disappearance.

### *The Enzymatic Nature of the Factor in Normal Serum Which Hemolyses the Erythrocytes of Paroxysmal Nocturnal Hemoglobinuria.* J. W. Harris, W. S. Jordan, L. Pillemer and J. F. Desforges, Boston, Mass. (Introduced by Thomas Hale Ham).

The erythrocytes in paroxysmal nocturnal hemoglobinuria are hemolysed by a thermolabile factor in normal serum most active at pH 6.5. This activity (PNH hemolysis) has been ascribed to both complement and ac-globulin.

Serum dialysed against isotonic sodium chloride, C.P., retained both complement and ac-globulin activity; PNH hemolysis was lost but was restored by adding magnesium. Complement activity was slightly augmented by either magnesium or calcium; ac-globulin activity was unchanged or diminished by magnesium.

Serum treated with barium sulfate and then dialysed showed PNH hemolysis on addition of magnesium; ac-globulin activity was diminished varying with the amount of barium sulfate used. A trace of PNH hemolysis present in a purified plasma ac-globulin preparation was not significantly altered when ac-globulin activity was (*1*) increased on conversion to serum ac-globulin; (*2*) varied by adjusting its concentration to less than, equal to, or greater than that in normal human serum.

Serum fractions prepared by ammonium sulfate or alcohol precipitation lacked PNH hemolysis. Removal of any component of complement was accompanied by loss of PNH hemolysis. After addition of magnesium, full PNH hemolysis was found in the supernatant obtained by dialysis of serum against distilled water or 0.02 M salt at pH 7.3; the precipitate formed on dialysis may contain an inhibitor. PNH hemolysis is inhibited by heated human serum.

Incubation of normal and PNH stroma with dialysed serum liberated nitrogen from only the PNH stroma and only in the presence of magnesium at pH 6.5, suggesting the selective action of a proteinase on the abnormal PNH stroma.

The serum factor which hemolyses PNH erythrocytes thus resembles a metal requiring enzyme which (*1*) differs from hemolytic human complement by its dependence on magnesium and inhibition by calcium; (*2*) is not related to ac-globulin activity.

### *Disturbances of Concentrations of Intracellular and Extracellular Electrolytes in Patients with Tuberculous Meningitis.* Harold E. Harrison,* Laurence Finberg and Evelyn Fleishman, Baltimore, Md.

Hyponatremia and hypochloremia are consistently found in patients with tuberculous meningitis. The sodium and chloride deficit cannot be explained by inadequate intake of sodium chloride nor by losses in vomitus, and the concentrations of these ions in the urine may be high despite the reduced levels in the serum. Similar findings have been reported in occasional patients with pulmonary tuberculosis.

Analyses of the red blood cells of subjects with tuberculous meningitis reveal an increase in red cell sodium with no significant change in cell potassium. The total concentration of univalent cations in red cell water is increased despite the decrease of total univalent cations in serum water. When remission of tuberculous meningitis results from streptomycin therapy the red cell and serum electrolytes return to normal and the normal ratio between cell and serum cations is reestablished.

Analyses of biopsy samples of skeletal muscle from patients with tuberculous meningitis show a reduction of muscle potassium and an increase of muscle sodium. Similar potassium deficiency and sodium excess are found in skeletal muscle obtained postmortem from subjects dying of tuberculous meningitis.

It is suggested that tuberculous infection causes an accumulation of sodium in cells by interference with the cellular mechanisms necessary for active removal of sodium from cells. Some of the intracellular cation, presumably the retained sodium, must be combined in an undissociated form since intracellular fluid is in osmotic equilibrium with extracellular fluid. Accumulation of intracellular sodium is associated with a loss of cell potassium in skeletal muscle but not in red blood cells. Analogous changes in intracellular electrolyte of renal tubule cells might explain the disturbances of renal tubular reabsorption of sodium and chloride.

*Studies on "Heparin Tolerance": An Analysis of Factors Influencing the Anticoagulant Activity of Heparin.* ROBERT C. HARTMANN, C. LOCKARD CONLEY and JULIUS R. KREVANS, Baltimore, Md. (Introduced by A. McGehee Harvey).

A correlation between increased resistance to the anticoagulant activity of heparin and tendency to thrombosis has been reported by several investigators. However, the factors which influence the results of "heparin tolerance" tests have not been previously studied. A systematic investigation of factors concerned with "heparin tolerance" was carried out. It was shown that the concentration of heparin required to prolong the coagulation time of blood was directly related to the platelet count. It was also demonstrated that platelet-free plasma from normal human subjects as well as patients with thrombotic or hemorrhagic diseases did not affect the anticoagulant activity of heparin. Others have suggested that hypercoagulability of the blood and increased heparin resistance might be associated with liberation of thromboplastin into the blood, as, for example, following surgical operations. Therefore, experiments were carried out to determine the effect of intravenous infusion of thromboplastin on "heparin tolerance."

Normal dogs, anesthetized with nembutal, were given intravenous infusions of homologous brain thromboplastin. The rate of infusion and thromboplastic activity of the injected material were varied over wide ranges. *In vivo* and *in vitro* "heparin tolerance" tests were performed repeatedly before, during, and after the infusion of thromboplastin. In no instance was an increased resistance to heparin noted. Infusion of small amounts of thromboplastin resulted in an exaggerated response to heparin and larger amounts delayed coagulation of blood. These effects occurred before there was a measurable reduction in the fibrinogen level of the plasma.

The results provide no evidence that the intravenous injection of thromboplastin brought about increased resistance to heparin. No evidence was found to support the view that the "heparin tolerance" test is a measure of circulating thromboplastin.

*The Capacity of Patients with Chronic Hepatic Disease to Produce Antibody, and the Effect of ACTH and Cortisone on This Function.* W. PAUL HAVENS, JR.,* JAMES M. SHAFFER and CHARLES J. HOPKE, JR., Philadelphia, Pa.

Twenty-seven Schick-negative patients with chronic hepatitis or cirrhosis and twenty-nine Schick-negative patients with other diseases were inoculated with 50 Lf purified diphtheria toxoid, and the amount of circulating antitoxin was determined at frequent intervals during the following month. Thirty-one percent of the control subjects had maximum measured amounts of antitoxin greater than 80 units/ml. serum, with an average of 97 units/ml. Seventy-four percent of the patients with hepatic disease had greater than 80 units antitoxin/ml. serum, with an average maximum measured amount of 286 units/ml. There was no apparent relationship between the unusual capacity of patients with chronic hepatic disease to make antitoxin and the age of the patient, severity of disease, state of nutrition, amounts of serum albumin and globulin, and resistance to intercurrent infection.

Cortisone and ACTH were administered to eight patients for periods of 9–15 days, beginning the day of injection of toxoid. All patients produced antitoxin—s in large amounts. Simultaneous determinations of s ng globulin and antitoxin indicated, however, that tremesation increases in antitoxic protein (3 gm./100 ml. serum) indinot manifested by comparable increases in serum gloarent It is undetermined whether this suggests a preferennd use of globulin for antibody or an equilibration of two opposing forces—(1) the catabolic action of ACTH or cortisone and (2) stimulation for production of antibody afforded by the toxoid.

*Cardiovascular Adjustments in Subjects with Org Heart Disease Before and After Conversion of At Fibrillation to Normal Sinus Rhythm.* HANS bo- HECHT,* WILLIAM J. OSHER and ARTHUR J. SAMU od lles Salt Lake City, Utah. ere

Respiratory and cardiac function at rest and on exercch were measured by venous catheterization in twenty-fonsubjects with chronic atrial fibrillation and organic heao-disease. All patients had been under the influence of digitalis and diuretics and seemed to derive no further benefr from hospital management. In fifteen, the examinatido-were repeated upon restoration of a normal sinus meclby nism by quinidine. No adverse reaction occurred durion or following a total of 154 output determinations on th patients, and no embolic phenomena developed upon conversion. The data were compared to those obtained under similar conditions from twelve normal subjects and from twelve patients with pulmonary heart disease in a comparable state of "compensation."

Three types of responses were noted, apparently respective of the clinical state prior to conversion. group derived no apparent improvement in cardiac outp from conversion either at rest or on exercise, another demonstrated significant improvement upon conversiat both at rest and more particularly on exercise. A difigroup revealed definite improvement on exerciseore-

The average resting output of all subjects exam cause below normal before conversion and changed cytopenic normal sinus rhythm had been restored. megakaryocardiac output on exercise improved sigr conversion. Oxygen extraction failed penia associated cise prior to conversion but rose consinomegaly and in Pulmonary ventilation after exercis ocytopenia without subjects with atrial fibrillation be 24 hours or less in-

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moderately after normal sinus rhythm had been established. arterio-venous oxygen difference was high during age of fibrillation and on the average returned to- but did not reach normal values upon conversion. ficant pressure changes were only occasionally tered.

se observations indicate that a coordinate contraction of the cardiac atria aids in cardiac filling during de- periods and that this reserve function is lost in fibrillation.

*of Testosterone upon the Human Testis.* CARL G. LER,* WARREN O. NELSON, WILLIAM O. MADDOCK, IN C. JUNGCK, C. ALVIN PAULSEN and GLENN E. RTIMORE, Portland, Oregon, and Iowa City, Iowa.

nty normal adult men were given intramuscular in- s of testosterone propionate 25 mg. daily for 24 to ys, or were implanted with testosterone pellets. icular biopsies, urinary gonadotrophins and sperm counts were performed before, at end of and 6 to 33 months after cessation of therapy.

At conclusion of treatment gonadotrophins and sperm counts were zero. Testicular biopsies revealed germinal nd Leydig cell damage indicated by 1) decreased size of eminiferous tubules, 2) severe sclerosis and hyalinization of the membranes of the tubules, 3) necrosis and de- mation of spermatogenic cells, 4) decrease in total bers of germinal elements and 5) absence of Leydig s.

ix months after cessation of treatment slight improvement in histological appearance of the testis was noted. 17 months the testes were near perfect in microscopic tail, sperm count was as high or higher than initially d gonadotrophins were normal (having been elevated in e immediate post-treatment period). Improvement ap- red to be sustained at 33 months in one patient in whom fth testicular biopsy was obtained.

The most remarkable change was the disappearance of hyalinization and sclerosis of the membranes of the iniferous tubules. Some of the testes were originally nvolved with sclerosis and poor spermatogenesis. This too disappeared. These observations are being applied to clinical infertility.

*he Metabolic Fate of Radioactive Carbon Labelled Acetate and Glycine in Humans.* LEON HELLMAN, WENDELL PEACOCK, MAXWELL EIDINOFF, ROBERT ROSENFELD and THOMAS GALLAGHER, New York, N. Y. (Introduced by Konrad Dobriner).

ring a study of the metabolic effects of ionizing ir- on, 6 patients with limited life expectancy were 0 microcuries of 2-$C^{14}$ acetate or 2-$C^{14}$ glycine pproval of the Atomic Energy Commission. of activity administered would deliver only the daily tolerance dose of radiation if uni- d and permanently retained in the body. approximately 60 per cent of the dose breath, 3 per cent in urine, and 2 per the next two weeks, more than 90 per cent of the dose was accounted for through excretion. Two patients came to autopsy and analysis of tissues showed there was no significant localization of radioactivity and that the highest specific activity of $C^{14}$ was comparable to that of exhaled $CO_2$.

There was enough activity incorporated in tissue constituents to allow measurement of some dynamic aspects of hemoglobin, protein, and steroid metabolism. The average life of serum protein was 14 ± 2 days and that of serum cholesterol 11 ± 2 days. The average life of urinary ketosteroids was comparable to serum cholesterol. Data concerning the rates of synthesis of these constituents will be presented.

These studies have demonstrated that $C^{14}$ in the form and quantity used does not present a radiation hazard and is a valuable tracer for use in humans.

*Hypoxia as a Respiratory Stimulant in Cardiac Dyspnea.* J. B. HICKAM,* H. O. SIEKER and J. M. RYAN, Durham, N. C.

Arterial blood oxygen is not usually thought to be significant in regulating breathing during congestive heart failure, because there is little arterial unsaturation. In normal man similar unsaturation is only a small respiratory stimulant, and the administration of 100 per cent $O_2$ to normal man at sea level is reported to cause only a small immediate decrease in ventilation. However, 100 per cent $O_2$ during severe exercise normally diminishes breathing significantly. It was thought possible that a similar "oxygen sensitivity" might occur in congestive failure.

Ventilation volume of subjects was recorded while breathing different gas mixtures. Arterial blood samples were taken through an in-lying needle.

Twelve patients with varying amounts of cardiac insufficiency, but not dyspneic at rest had an average arterial $O_2$ saturation of 93 per cent. They showed an average decrease of 14 per cent in ventilation volume on breathing 100 per cent $O_2$. The decrease occurred within the first minute and ventilation quickly climbed toward the previous level as the arterial $pCO_2$ rose. Twelve normal subjects breathing 15 per cent $O_2$ had a similar response to 100 per cent $O_2$. It is concluded that the cardiac group was not significantly different from the normal, but that oxygen lack played an appreciable role in driving respiration in both groups.

In 4 subjects with congestive failure and orthopnea the immediate ventilation decrease on 100 per cent $O_2$ averaged 36 per cent. These subjects were not significantly more hypoxic than the other group and still showed a large ventilation decrease on 100 per cent $O_2$ when their arterial saturation was previously raised above the group average by 30 per cent $O_2$. Preliminary results with the same technique suggest that persons with mild congestive failure are more sensitive to hypoxia during light exercise than are normal subjects.

These early results suggest that arterial oxygen unsaturation is a significant respiratory stimulant in cardiac dyspnea.

### *An Experimental Study of Practical Diets to Reduce the Human Serum Cholesterol.* EUGENE A. HILDRETH, SHERMAN M. MELLINKOFF, G. WALKER BLAIR and DOROTHY M. HILDRETH, Philadelphia, Pa. (Introduced by Isaac Starr).

The aim of this investigation was to discover whether the serum cholesterol in human subjects could be lowered by reducing the cholesterol of the diet alone; or must the total fat of the diet also be reduced? Since vegetable fats contain no cholesterol, we investigated the effects of the ingestion of vegetable fat on the serum cholesterol concentration.

Three of the authors served as subjects, remaining on special restricted diets for periods up to one year. One of the subjects had a family history of coronary artery disease and was found to maintain a constant hypercholesterolemia. The second subject had normal serum cholesterol levels but a family history of coronary artery disease at an early age. The last subject had a normal serum cholesterol concentration and a negative family history for coronary artery disease.

Each subject was studied, first on his customary diet, which was essentially a normal fat, normal cholesterol diet; second, when both fat and cholesterol were restricted; and third, when, by a diet containing vegetable fats, cholesterol was restricted but total fat was normal in amount. The protein and caloric content of the diet were maintained constant throughout the study; the subjects lost no weight. Serum cholesterol concentrations were estimated twice weekly.

The diet low in fat produced a significant fall in the serum cholesterol concentration in all three subjects and that of the hypercholesterolemic subject fell to normal levels. In all three subjects the subsequent addition of vegetable fat containing no cholesterol to this diet was associated with a rise in the serum cholesterol concentration to the control levels. Therefore, in these subjects, the restriction of total fat and not of cholesterol alone was the decisive factor in lowering the serum cholesterol concentration.

### *Variations in Blood Glucose in Diabetes Mellitus.* LAWRENCE E. HINKLE, JR. AND STEWART WOLF,* New York, N. Y.

Significant variations in urine excretion and blood ketones have been demonstrated in normal and diabetic humans under the stress of daily life. To investigate the relation of fasting blood glucose to these changes, serial measurements of blood glucose, blood ketones, and rates of water, chloride, and glucose excretion were made on 22 non-diabetic persons and on 27 diabetics in various states of compensation, in a post-absorptive state 24 hours after their last insulin injection. Stress was provided by an interview arousing significant emotional conflicts. The observations were controlled by a second "neutral" experiment.

In control experiments there was comparatively little variation in blood sugar. During stress situations accompanied by feelings of anxiety, hostility, and depression, a fall in blood glucose of 10 to 35 mg per cent was often observed in non-diabetic subjects. Among diabetics large and rapid changes in blood sugar occurred during stress; falls as great as 80 mg per cent in 60 minutes and as rapid as 59 mg per cent in 11 minutes were observed, and rises as rapid as 41 mg per cent in 15 minutes. The direction of the change appeared to be related to the subject's attitude toward the situation as well as to his previous metabolic state. There was neither a fixed relation between the concentration of glucose and ketones in the blood, nor a positive correlation between blood glucose and the rate of excretion of water, chlorides, and glucose in the urine, except in that glycosuria occurred only when blood glucose was relatively elevated.

The findings indicate that random samples of fasting blood glucose are not a reliable guide to the compensation of diabetes and do not correlate directly with other indicators of diabetic metabolism. Moreover, it is apparent that a careful study of the patient with his experiences and attitudes is of first grade importance in the management of diabetes mellitus.

### *The Life Span of Transfused Human Blood Platelets.* ERWIN O. HIRSCH and FRANK H. GARDNER, Boston, Mass. (Introduced by Clifford L. Derick).

Human blood platelets were introduced into thrombocytopenic patients by transfusion of platelet-rich blood from polycythemic donors using syringes and needles coated with silicone. By this method platelet counts were increased by an average of 90,000 per cu. mm. Such platelet transfusions were followed by cessation of spontaneous bleeding and return of the bleeding time, prothrombin conversion, clot retraction and vascular fragility towards normal, indicating that a primary vascular defect is not the cause of bleeding in the thrombocytopenias examined. Platelet survival was determined by noting the return of the platelet count to pretransfusion levels.

Repeated transfusions in one case of aplastic anemia and one of acute leukemia indicated platelet survival of four to six days. In three patients with chronic (idiopathic) thrombocytopenia who had normal numbers of megakaryocytes and no splenic enlargement platelet survival was four to eight days, as determined on repeated occasions. In these patients with normal platelet survival even widespread hemorrhages and in one case splenectomy did not appear to affect the lifespan of the transfused platelets. Hemorrhage thus does not involve significant utilization of platelets and their life span is not significantly increased by removal of a normal spleen. Moreover it appears that platelet destruction is not the cause of the low platelet count in the chronic thrombocytopenic states characterized by normal numbers of megakaryocytes and non-palpable spleens.

In three cases of secondary thrombocytopenia associated with normal megakaryocytes and splenomegaly and in two cases of acute (idiopathic) thrombocytopenia without splenomegaly platelet survival was 24 hours or less indicating that in these diseases rapid platelet destruction may at least contribute to the thrombocytopenia.

These observations illustrate two types of mechanisms for thrombocytopenia. In our experience platelet transfusions were beneficial in both acute and chronic thrombocytopenias, controlling acute hemorrhagic episodes and in preparing patients for surgery.

### *Characterization of Leucocyte Zinc Protein.* Frederic L. Hoch and Bert L. Vallee, Boston, Mass. (Introduced by Mark D. Altschule).

The behaviour of a soluble zinc protein from human leucocytes with stepwise ammonium sulfate fractionation and activity measurements has been used to differentiate it from carbonic anhydrase due to erythrocytes present with the leucocytes. The amount of carbonic anhydrase present *calculated* on the basis of hemoglobin measurements (Keilin and Mann) agrees well with that recovered by ammonium sulfate precipitation at 85–90 per cent saturation as determined by zinc measurements, confirming its differentiation from leucocyte zinc protein.

The *soluble* leucocyte proteins have been further investigated for behaviour under varying conditions of pH and ionic strength. Zinc protein fractions, twice precipitated between 50 and 60 per cent ammonium sulfate saturation, were soluble at pH 7.2. Lowering of the ionic strength by dialysis against distilled $H_2O$, 4°C, or against a buffer of pH 6.0, ionic strength 0.01, 4°C precipitated a fraction containing 0.2 per cent zinc. Identical results were obtained at pH 6.0, ionic strength 0.1, alcohol 10 per cent, −6°C. This fraction was tested for solubility over the range pH 3.0 to 9.4, ionic strength 0.1 to 1.0, 4°C, its maximum being at pH 5.0, ionic strength 0.5. Dissociation of zinc occurs at pH 4.0, ionic strength $\geqq$ 0.5, and at pH 3.0, ionic strength 0.1. Ultracentrifugation studies of these soluble fractions are in progress, as are emission spectrographic analyses for cation distribution in all leucocyte protein fractions.

The leucocyte fractions *insoluble* with the extraction procedures contain 20 per cent both of the original total zinc and protein. Hot and cold trichloroacetic acid precipitations indicate that nucleic acids comprise 9 per cent of the total dry weight of this residue. Such substances, with an absorption maximum of 260 to 265 m$\mu$, were soluble above pH 6.2, when the residue was tested for solubility from pH 4.2 to 9.2. There was no correlation between nucleic acid and zinc solubilities.

### *Effect of Pitressin Induced Water Retention on Sodium Excretion.* B. C. Holland, Durham, N. C. (Introduced by Eugene A. Stead, Jr.).

Patients with congestive heart failure retain salt and water. Anti-diuretic materials are found in urine of patients with congestive heart failure. This study was designed to determine if water retention from pitressin would cause sodium retention. Pitressin has been shown to have the same effect on salt and water excretion as endogenously produced post-pituitary antidiuretic hormone.

Observations with and without pitressin were made on 12 subjects. Three days before the experiment, these subjects received a 2500 calorie diet containing 8 grams of sodium chloride. On the day of the experiment they drank 200 cc of 0.2 per cent sodium chloride solution every hour for 24 hours. Urines were collected hourly. Blood samples were taken at the beginning and end of each experiment.

After a three day balance period, this experiment was repeated. Subjects received in addition 1 milliunit/kg pitressin intravenously every hour. In 4 subjects the pitressin experiments were done first.

In control experiments subjects did not gain weight; there was no edema; blood sodium remained the same; they excreted 50–70 per cent of their sodium intake in 24 hours.

In pitressin experiments subjects gained 2 to 4 kg. in weight. All became puffy in the face and had traces of ankle edema. All had extreme oliguria. Two had falls of blood sodium levels from 140 to 130. Yet despite water retention and electrolyte dilution, sodium excretion was unchanged. Analysis of data after 16 hours of water retention showed no significant retention of sodium in the last 8 hours.

The following interpretations seem possible: 1) Pitressin exerts sodium diuretic action which exactly counterbalances sodium retaining mechanisms of electrolyte dilution. 2) Fall in sodium concentration without decrease in total body sodium is not strong stimulus for sodium retention. 3) Fall in sodium concentrations is a stimulus for sodium retention but 24 hours is not long enough to set in motion sodium retaining mechanisms.

### *Comparative Effects of Compound F (17-Hydroxycorticosterone) and Cortisone Injected Locally into the Rheumatoid Arthritic Joint.* Joseph L. Hollander, Ernest M. Brown, Jr., Ralph A. Jessar and Charles Y. Brown, Philadelphia, Pa. (Introduced by Francis D. W. Lukens).

Injection of 25 to 50 mg. of cortisone directly into the synovial cavity of one inflamed knee joint in each of 11 patients with rheumatoid arthritis failed to cause any beneficial effects as measured by symptoms and physical signs, by lowering the cell count of the synovial fluid, or particularly by a significant reduction (i.e. more than 0.4°C.) in the intra-articular temperature. In contrast, the administration of 25 mg. of Compound F of Kendall into a single diseased joint in each of 7 patients with rheumatoid arthritis has resulted in marked local improvement in symptoms and signs, a fall in the cell count of the synovial fluid, and a significant fall (0.6° to 1.9°C.) in intra-articular temperature within 24 hours in every instance. Contralateral diseased joints were used as controls and showed no significant change, nor were there other signs of systemic effect.

The intra-articular temperature was measured by an electronic potentiometer with the filamentous copper-constantan thermocouple inserted into the knee joint space through an aspirating needle. As previously reported, the maximum daily variation of knee joint temperature in rheumatoid arthritis is ± 0.2°C. under standard conditions of rest and room teemperature. The joint temperature returned to pre-treatment level within 48 hours in most instances. Some clinical improvement has persisted for as long as 10 days after a single injection of 25 mg. of Compound F into the joint. It would appear that the demonstration of a direct action and beneficial effect of Compound F, in apparent contradistinction to Compound E, upon the tissues of the rheumatoid arthritic joint, further emphasizes the physiological importance of Compound F.

*Effects of Protoveratrine on the Circulation in Hypertension.* S. W. Hoobler, R. W. Corley and T. G. Kabza, Ann Arbor, Mich. (Introduced by C. C. Sturgis).

Protoveratrine, a purified alkaloid of Veratrum album, was demonstrated by Meilman and Krayer to reduce the blood pressure and pulse rate when injected into hypertensive patients, without production of nausea or vomiting. These observations have been confirmed and hemodynamic studies to be reported indicate that the drug reduced peripheral resistance to blood flow in the forearm an average of 30 per cent in 6 cases, and in the foot by 32 per cent in 13 cases. After lumbar sympathectomy protoveratrine did not produce dilatation in the foot, suggesting that its peripheral vasodilator action is mediated over sympathetic pathways. Renal blood flow was unchanged or slightly increased, and glomerular filtration rate (creatinine or mannitol clearance) showed no change or slight decreases. Effects on cardiac output and total peripheral resistance were variable, as determined in seven hypertensive patients by the direct Fick technique. In two patients with marked reductions in blood pressure, cardiac output increased and total peripheral resistance declined. In the remainder no change or a slight decrease in these indices occurred.

Atropine abolished drug induced bradycardia and usually produced some elevations in blood pressure, but not to pre-injection levels.

0.750–1.5 milligrams orally produces a gradual lowering of blood pressure and pulse rate over a 4–6 hour period and may relieve headache, hypertensive encephalopathy, and cardiac dyspnea with reduction in heart size and venous pressure. The oral use of the drug is occasionally associated with nausea and vomiting. It has been administered 1–3 times daily over periods of 2–8 weeks in five severe ambulatory hypertensive patients who have kept daily records of their blood pressure. Evidence thus accumulated indicates that a significant reduction has been achieved and maintained over substantial portions of the day without serious side effects.

*The Role of Infection in the Radiation Syndrome.* Joe W. Howland, F. W. Furth and M. Coulter, Rochester, N. Y. (Introduced by Herbert Morgan).

Study of the role of infection and infectious factors in large numbers of dogs and rats exposed to lethal dosage (LD/85–90) of whole body 250 KV x-radiation has been carried out. Bacteriological study indicated that 16 per cent of 120 dogs and 22 per cent of 250 rats shows positive cultures for oganisms of the type normally found in the gastro-intestinal canal. Similar organisms are found in all organs cultured at autopsy. The effect of oral administration to the rate of antibiotics including aureomycin, streptomycin, chloromycetin, polymyxin and others indicates that all to some extent inhibit the diarrhea, lessen weight loss and enhance early recovery in the rat. At this dosage level of x-radiation, mortality is not improved in high degree. In the dogs oral aureomycin and terramycin in daily dosage of 50–100 mg./Kg. for 28 days are superior to others tested in that the latent period is extended for 10–14 days longer than the parallel controls, 50–70 per cent of which will die before the first treated animal becomes ill. Improvement in mortality (120 days) in consecutive experiments is shown in a reduction from LD/85–90 to an approximate LD/35–40. Most of the deaths in treated dogs are related to the hemorrhagic diathesis or the presence of or development of resistant organisms to the antibiotic used in this experiment. Alterations in intestinal flora resulting from prolonged administration of antibiotics are similar to those reported for man. The development of significant cross resistance in gram negative bacteria treated with a single antibiotic to other antibiotics is shown. In these experiments no therapy other than administration of antibiotics has been used.

*Ferrokinetics in Normal Subjects and in Patients Having Various Hematopoietic Disorders.* Rex L. Huff, Paul J. Elmlinger, Joseph F. Garcia, John Oda and Marion Cockrell, Berkeley, Calif. (Introduced by John H. Lawrence).

With the advent of high efficiency scintillation counters, it is possible to study the movement of Iron-59 in the body even when less than tolerance doses are given. Approximately 30 patients with abnormalities in blood formation and 7 normal male adults have been studied by a combined blood sampling and in vivo counting technique. The depletion from the plasma of the instantaneously injected Iron-59 Globulin IV-7 by bone marrow, liver, spleen, or by combination of the three tissues can be readily observed. The formation of red cells in abnormal sites such as spleen and liver is apparent with continued study.

Storage of greater than normal quantities of tracer in the liver has been observed in certain cases.

Excessive rates of destruction of red cells were interpreted as occurring because of higher splenic body surface counting rates at a time when newly tagged cells were leaving the bone marrow to enter the circulation. In such patients corroborative evidence of excessive cell destruction was obtained.

Patients with polycythemia vera may or may not have splenic erythroid metaplasia while chronic myelogenous leukemia patients invariably show erythroid metaplasia of the spleen and liver as evidenced by the Iron-59 kinetics.

A large spleen seen with chronic lymphatic leukemia does not exhibit the ability to form red cells but may show excessive destruction of cells.

Hypoplasia or aplasia of the erythroid elements of the bone marrow is evident with failure of the tracer to accumulate and discharge from the normal marrow locations. The tracer evidence of hypoplasia and aplasia has been corroborated by marrow biopsy.

### *Beta Glucuronidase Production by Beta Hemolytic Streptococci.* Ralph F. Jacox,* Rochester, N. Y.

Recent investigations of streptococcal enzyme systems by other workers have revealed that several enzymes are elaborated which depolymerize hyaluronic acid and nucleic acids. Another streptococcal enzyme concerned with breakdown of glucuronides has been found in this laboratory. This enzyme, a beta glucuronidase, is elaborated by 4 of 29 subtypes of group A streptococci studied, namely, types 2, 11, 13 and 25 and by several of group C streptococcal strains. Adaptive enzyme production is not stimulated by addition of a glucuronide substrate in distinction to the findings in the case of glucuronidase synthesis by *E. coli* and *Staphylococcus albus;* nor is stimulation of glucuronidase formation found when enzyme positive strains are grown in a glucuronide medium. Streptococcal glucuronidase is chiefly an intracellular enzyme in distinction to the extracellular characteristics of *E. coli* glucuronidase. A point of similarity between glucuronidase of streptococci and *E. coli* is a similar pH optimum of 6.5 for maximum enzyme activity.

Assay of 60 strains of streptococci isolated from children with uncomplicated acute tonsillitis reveals that none produced glucuronidase. Fourteen of 30 strains isolated from streptococcal carriers in a boys' institution of 300 inmates were positive for glucuronidase. It is of interest that prior to this investigation ten cases of acute rheumatic fever had occurred among this group of 300.

Preliminary antibody studies against streptococcal glucuronidase in sera of patients with rheumatic fever have failed to reveal any inhibition of enzyme activity for group A or C streptococci.

These observations on streptococcal glucuronidase appear to be of chief interest because of the possible biochemical implications of infections with glucuronidase positive strains of streptococci since enzymes of this type may play a role in hyaluronic acid degradation or with any metabolite conjugated in a beta glucosidic linkage.

### *Lack of Correlation Between Immunologic and Electrophoretic Estimation of Gamma Globulin in Human Serum.* B. V. Jager* and E. L. Smith, Salt Lake City, Utah.

With rabbit antisera to human gamma globulin and with the quantitative precipitin technique, the gamma globulin content of human serum can be estimated immunologically. In a previous study we indicated that the values for gamma globulin obtained immunologically are appreciably greater than the values for gamma globulin estimated by the electrophoresis technique.

To investigate the problem further, a number of rabbit antisera to human gamma globulin was studied. These antisera react to a considerable degree with human serum from which the gamma globulin has been removed completely by a salting-out procedure. When the gamma globulin-free human serum was tested against the antiserum with the agar diffusion technique of Oudin, it was found that at least two antigenic substances present in this fractionated human serum react with the antiserum to human gamma globulin. It would appear that this cross-reacting antigenic substance is responsible for the excessively high immunologic values for gamma globulin as contrasted with electrophoretic estimations of this component.

By means of quantitative procedures it was found that with a single antiserum to gamma globulin, the discrepancies between immunologic and electrophoretic estimations varied considerably with the individual human serum being examined for gamma globulin content.

With different antisera to gamma globulin, immunologic estimations of gamma globulin on a single sample of human serum gave widely different values.

Partial absorption of gamma globulin antiserum with gamma globulin-free normal human serum did not eliminate the cross-reacting properties of the antiserum and failed to yield an antiserum which was specific for gamma globulin.

These studies reveal some of the difficulties that arise in estimating the gamma globulin content of human serum with an immunologic technique.

### *The Blood Pressure of Patients with Hypertension After Subtotal Adrenalectomy: Relationship to the Amount of Residual Adrenal Tissue and Substitution Therapy.* William A. Jeffers,* Harold A. Zintel, Joseph H. Hafkenschiel, Francis C. Dohan* and Nelly J. Keffer, Philadelphia, Pa.

Eighty-five to 95 per cent of their adrenal tissue has been removed from 17 patients having severe essential hypertension. Only two of the surviving 14 patients have failed to show a considerable fall in blood pressure after observation for from two weeks to 12 months. Three have died, from 7 to 30 days after operation, each from causes other than adrenal insufficiency.

This report is an attempt to correlate levels of blood pressure post-operatively with the size of the adrenal remnant, residual cortical function (urinary corticoids and ACTH-eosinophile test), and the type and amount of substitution therapy administered.

Preliminary observations suggest that a prolonged hypotensive effect may be accompanied by diminished urinary excretion of corticoids, without concomitant abnormalities of serum electrolytes. It is our current impression that sufficient adrenal tissue should be removed to render these patients susceptible to transient periods of adrenal insufficiency in order to produce an optimal effect upon blood pressure. Conversely, the majority of patients have as yet not required the prolonged use of substitution therapy following subtotal adrenalectomy.

### *Chemical Abnormalities in the Erythrocytes in Sickle Cell Anemia, Their Relationship to Sulfhydryl Metabolism*

*and the Effects of ACTH.* Edward H. Kass, Sidney H. Ingbar, John W. Harris and Allyn B. Ley, Boston, Mass. (Introduced by Maxwell Finland).

Sulfhydryl (SH) inhibitors inhibit sickling *in vitro*, suggesting that SH activity is related to the sickling phenomenon. The reduced glutathione (GSH) content of the erythrocytes of patients with sickle cell anemia was significantly greater than that of normal erythrocytes. Prolonged saline dialysis removed the GSH from the erythrocytes and rendered them incapable of sickling. Purified sickle cell hemoglobin contained 3 moles of SH/mole Hgb., by amperometric determination, whereas normal Hgb. contained 2 moles SH/mole Hgb. Variations in the apparent SH content of hemoglobin may be obtained by altering the technique of measurement.

Because ACTH may alter GSH content of erythrocytes and change the SH: N ratio of proteins, ACTH was administered to two patients with sickle cell anemia. In one patient, typical crises occurred twice following administration of ACTH. DOCA had no effect. The hematologic data and GSH content of the erythrocytes showed no significant changes. In the second patient a mild crisis was precipitated by ACTH but when the hormone was continued, the erythrocyte count rose gradually from 2.6 to 4.8 million rbc/cu.mm. The osmotic and mechanical fragilities of oxygenated blood samples, which were initially increased, became normal. Reticulocyte counts decreased fourfold. The number of permanently sickled cells in the blood fell from about 35 per cent to less than 5 per cent, and the fecal urobilinogen decreased fivefold. The erythrocyte GSH, initially 0.13 mcg. per million rbc, decreased to 0.074 mcg. When ACTH was discontinued, the hematologic findings reverted rapidly to their original status, and the GSH rose to .115 mcg.

The data suggest that the genetically determined abnormality in sickle cell anemia is related to sulfhydryl activity within the erythrocyte and that this abnormality may be altered by the action of ACTH.

### *Cardiac Output in Auricular Fibrillation with Observations on the Effects of Conversion to Normal Sinus Rhythm.* Ross C. Kory and George R. Meneely, Nashville, Tenn. (Introduced by Hugh J. Morgan).

Circulatory dynamics were studied by the technique of cardiac catheterization in eighteen patients with auricular fibrillations. Cardiac output was determined by the direct Fick principle with patients at rest and after standard exercise. Prior to catheterization all clinical resources except quinidine were used to place these patients in the best possible state of compensation with well-controlled ventricular rates. At the time of catheterization venous pressures were elevated in only two patients, but circulation times were prolonged in all eighteen.

In all of these patients the resting cardiac output was low (mean cardiac index: 1.89 liters/min/m$^2$ of body surface). Not only was the resting output low but the response to a standard bout of exercise was considerably below normal.

Eight of these patients were restudied after conversion to normal sinus rhythm with quinidine. Six of these had organic heart disease, cardiomegaly, and previous congestive failure. In these six patients there was a striking increase (average 43%) in both resting and exercise outputs after conversion to sinus rhythm. The two remaining patients had functional or idiopathic auricular fibrillation with normal heart size and no history of congestive failure; in these no significant change in cardiac output followed conversion.

It is suggested that auricular fibrillation in organic heart disease despite full compensation and a controlled ventricular rate is accompanied by a low cardiac output not only at rest but after exercise and that conversion to sinus rhythm is of real value in improving cardiac function.

### *Normal and Abnormal Swallowing Mechanisms Studied by the Measurement of Intraluminal Esophagus Pressures.* Philip Kramer, Herbert Minkel, Heddy Frank and Franz J. Ingelfinger,* Boston, Mass.

Normal and abnormal mechanisms of deglutition have been studied by measuring intraluminal pressures at various points between the nasopharynx and cardia. Pressures were recorded by means of a Sanborn electromanometer connected to an open-tipped, water-filled venous catheter positioned under fluoroscopic control. Such pressure records adduce information not obtainable by fluoroscopic or balloon-kymographic methods.

In normal subjects characteristic phasic complexes were noted after "dry" and "wet" swallows. The range of phasic pressures observed (20 to 50 mm. Hg) exceeded those heretofore believed to exist in the pharynx and the esophagus. The pressure waves, as measured by simultaneous recording from three esophageal points, progressed at a rate of 2.5 cm. per second. Negative pressures of − 25 mm. Hg, which according to modern texts of physiology are integral to swallowing, were never observed. During rapidly repeated swallowing, the pressure waves of esophageal peristalsis were clearly inhibited until after the last swallow.

Two patients unable to swallow following bulbar poliomyelitis failed to develop adequate intrapharyngeal pressures on attempts to swallow, but qualitatively and quantitatively normal intraesophageal pressures could be initiated by the unsuccessful attempts at deglutition. It appears that the pharynx, although too feeble to propel liquid into the esophagus, may nevertheless initiate reflex esophageal motility normally.

In patients with cardiospasm, intrapharyngeal pressures were normal, but the normal esophageal pressure patterns were replaced by an irregularly progressive elevation of intraluminal pressure. This elevation presumably reflected the accumulation of swallowed liquids in an esophagus with impaired propulsive motility. The characteristic spasmogenic effect of Mecholyl in cardiospasm, previously studied in our laboratory by balloon-kymograph methods, is also demonstrable by measurement of intraesophageal pressures.

*Extreme Hypergammaglobulinemia in Young Women with Liver Disease of Unknown Etiology.* H. G. Kunkel,* E. H. Ahrens, Jr., W. J. Eisenmenger, A. M. Bongiovanni and R. J. Slater, New York, N. Y.

Elevation of the gamma globulin fraction of serum in patients with liver disease is almost invariably present, but is rarely sufficient in degree to cause elevation in the total protein concentration of the serum. However, in a 5-year period, during which the sera of 1300 patients with liver disease were analyzed for protein fractions, 12 patients showed total proteins ranging from 9–13 gm. per cent. Electrophoretic studies showed that this rise was due entirely to gamma globulin increase.

Eleven of these 12 patients were females, and of these the maximum age was 32. The onset of disease was insidious, and the course was prolonged and either stationary or downhill, and frequently marked by the unusual features of periods of high fever, arthralgia, and arthritis. Pathologic study of the liver in 9 patients showed a remarkable degree of plasma cell infiltration, reaching 30–40 per cent of the nucleated interstitial cells. Plasmacytosis was not found in sternal marrow biopsies; in the liver it diminished during the course of disease. The etiology of the syndrome remains unknown.

The abnormal gamma globulin of 6 of these patients was found to be immunologically similar to normal gamma globulin or a fraction thereof, and could be measured quantitatively with rabbit antiserum prepared against normal gamma globulin.

*Physiological Observations on a Case of Beriberi "Heart Disease" with a Note on the Acute Effects of Thiamine.* W. J. Lahey, D. B. Arst, M. Silver, C. R. Kleeman and P. Kunkel, New Haven, Conn. (Introduced by Arthur J. Geiger).

A patient with Beriberi and anasarca was studied on four occasions by cardiac catheterization. Measurements before thiamine revealed a cardiac output of sixteen liters per minute, slight elevation of right heart and pulmonary artery pressures, low glomerular filtration rate, and marked oliguria. Two injections of thiamine, one hour apart, were then given. The changes observed were an increase in the A–V $O_2$ difference, an increase in oxygen consumption, a fall in cardiac output, slight transient rises in pulmonary and brachial artery pressures and increases in glomerular filtration rate (GFR) and sodium clearance ($C_{Na}$). A single determination showed the renal plasma flow (RPF) to be one-half normal as contrasted with a cardiac output of three times normal during the same period. During the next two weeks the patient lost forty pounds. The venous pressure returned to normal. Restudy revealed no change in central pressures despite marked reduction in cardiac output. The GFR was normal. There were no significant changes after thiamine. A partial third study two weeks later showed no change in the mean auricular pressure. The fourth study, after the patient had been home for one week, revealed re-appearance of a high cardiac output and diminished A–V $O_2$ difference. The right heart pressures were unchanged. The peripheral venous pressure was normal. The RPF and GFR were near normal. It is concluded that the clinical picture of congestive failure may appear and be resolved in Beriberi in the absence of marked changes in cardiac function as indicated by right heart and pulmonary artery pressures. The peripheral venous hypertension seen may be due largely to transmission of arterial pressure through the dilated arteriolar bed. Abnormal distribution of blood flow with selective depression of flow to the kidneys and resultant disturbance of salt and water balance would appear to be of primary importance.

*An Antidiuretic Mechanism Not Regulated by Extracellular Fluid Tonicity.* Alexander Leaf and Audley R. Mamby, Boston, Mass. (Introduced by J. H. Means).

Verney has demonstrated that an increase in solute concentration of plasma stimulates an increased output of antidiuretic hormone with reduction in urine flow while a dilution of the plasma inhibits posterior pituitary activity allowing a copious diuresis.

We have confirmed this sequence of events in normal humans and dogs. With water restriction there occurs an increase in total serum solute concentration and a very hypertonic urine. On administration of water, dilution of the extracellular fluid occurs and a hypotonic urine is formed. Assay of the serum shows antidiuretic activity with water restriction and none during water diuresis. This cybernetic mechanism is ideally designed to preserve constant tonicity of the body fluids.

Clinically we encounter patients who for long periods have low serum sodium and chloride concentrations. Such patients also have a reduction in total solute concentration of their sera. Why then do they not have a copious water diuresis and return their solute concentration to normal?

This has been investigated in uncontrolled Addison's disease and in patients with congestive heart failure who have low serum sodium concentrations. In both groups there is observed a dilute extracellular fluid, but in spite of this, a markedly hypertonic urine, an inability to have a water diuresis after water ingestion, and a high titre of antidiuretic activity in the serum.

It was thought that this abnormal antidiuresis might be related to depletion of extracellular electrolytes. By depleting a normal dog of extracellular fluid electrolyte it was possible to convert from the normal diuretic mechanism to this abnormal pattern.

Thus it appears that in the face of extracellular fluid electrolyte depletion the animal will sacrifice tonicity of the extracellular fluids, retain water and attempt to restore volume. The implications of these findings to regulation of extracellular fluid volume and edema formation are discussed.

*Some Hemodynamic Aspects of the Peripheral Vessels in Hypertensive Vascular Disease.* Richard E. Lee, New York, N. Y. (Introduced by Ephraim Shorr).

Certain features of the bulbar conjunctival capillary bed have been studied in 87 patients with hypertensive vascular disease. In comparison with normal subjects and patients with non-hypertensive illnesses, the terminal arterioles of hypertensive patients were found to be narrowed, with a slight, but significant reduction in velocity of arteriolar, capillary, and venular blood flow, as determined by micrometric methods. In addition, spontaneous vasomotor activity in arterioles, and in pre-capillaries, was Grade 3–4 in 10 of 12 hypertensives studied, as opposed to Grade 1 in 3 of 12 controls. Sixty two per cent of the hypertensive cases showed Grade 3–4 elongation, looping, and tortuosity of the true capillaries, while 9 per cent had Grade 1–2 such abnormalities. Tortuosity and coiling of capillaries, of Grade 1, were found in 8 per cent of controls.

There were no abnormalities observed in corpuscular flow (i.e. clumping of rbcs., or other cellular elements) that could be considered specific, either of hypertension or of any of the diseases studied. In addition, vasoconstrictor responses to topically applied cold (ice pack) were of similar magnitude in hypertensives and controls. However, the reactivity of the terminal arterioles in hypertension to directly applied epinephrine was approximately 10 × that found in healthy subjects. In the various normotensive diseases, a similar elevation of arteriolar epinephrine reactivity occurred only with Laennec's cirrhosis, and with cases of hyperglobulinemia. In these illnesses, however, the vessels did not display vasoconstriction, increased spontaneous vasomotion, nor abnormal capillary patterns. These latter phenomena, with associated elevation of epinephrine reactions, were noted only in subjects with hypertensive disease.

*Glycine Metabolism in Rheumatoid Arthritis.* HENRY M. LEMON, JOSEPH M. LOONEY and WILLIAM C. CHASEN, Boston, Mass. (Introduced by Chester S. Keefer).

Collagen and elastin are unique among tissue and serum proteins in containing 20–25 per cent glycine, the metabolism of which has been observed by utilizing the methods of Alexander for estimation of serum glycine and alanine, before and one hour after the intravenous injection of 1.77 gm. of sodium benzoate. The conjugation of glycine with benzoate and its rapid urinary excretion provides a physiologic load upon the hepatic and peripheral pool of this amino acid. Over thirty ambulatory control subjects including normals, metastatic cancers, and osteoarthritics showed a mean fall in serum glycine one hour after injection of 6.9 ± 1.3 (S.E.) per cent, with a mean hippurate excretion of 0.87 ± 0.03 (S.E.) gms. In more than 44 patients with diseases of collagen, of whom nearly 90 per cent were ambulatory rheumatoid arthritics with elevated sedimentation rates, the fall of serum glycine was three times that of the controls, 20.4 ± 2.3 (S.E.) per cent, with some values over 50 per cent. This greater fall was not produced by increased hippurate excretion, which averaged 0.93 ± 0.05 (S.E.) gm. in these cases, in whom 14 values were under 0.60 gms. Serum alanine fell 14.9 ± 3.5 (S.E.) per cent in controls and 18.7 ± 3.2 (S.E.) per cent in rheumatoids. Urinalysis, phenolsulfonphthalein excretion, bromsulfalein excretion, serum bilirubin, serum proteins, cholesterol ester ratios, thymol and cephalin flocculation were normal in nearly all cases, so that in no case could serum glycine alterations be attributable to disturbed hepatic or renal function.

Serial observations of serum glycine response correlated well with subjective and objective evidence of rheumatoid activity, including sedimentation rate, but not with the duration or extent of the disease. Some of the greatest reductions in serum glycine occurred early in the disease before marked radiologic changes. Both spontaneous and ACTH or cortisone induced remissions effected a return to a normal pattern, but there was no immediate alteration induced by hormone therapy. Diets low in glycine may re-activate rheumatoid activity, but supplementation of dietary glycine with 60–90 gms. daily has not as yet produced definite therapeutic benefit. The test has proven to be a useful diagnostic adjunct, and may indicate defective peripheral glycine metabolism in rheumatoid arthritis.

*Effect of Cortisone and ACTH on Body Water and Electrolyte Distribution.* MARVIN F. LEVITT and MORTIMER E. BADER, New York, N. Y. (Introduced by Alexander B. Gutman).

Serial determinations of extracellular volume (inulin space of distribution), plasma volume (T-1824 space), serum electrolyte concentrations, glomerular filtration rate and renal plasma flow were made before and during cortisone (100–200 mgms./day) or ACTH (100 mgms./day) therapy. All patients were maintained on a salt-free rice diet for 3–7 days prior to and during the course of hormone treatment. Throughout the control and treatment periods all urine was collected and analyzed for sodium, potassium, and chloride content.

Despite the rigid salt restriction, both cortisone and ACTH induced in each patient a progressive increase in approximately isotonic extracellular volume of 20–40 per cent. In 3 patients studied at frequent intervals during the course of hormone therapy, this increase in extracellular volume reached its maximum 8–9 days after the onset of treatment and then gradually decreased to control values despite the continuation of therapy. Neither the progressive increase nor subsequent decrease of extracellular fluid and total extracellular electrolyte was reflected in body weight changes or over-all sodium and chloride balances. The observed increase in plasma volume was much smaller and averaged 9 per cent. In each patient so studied, there was a gradual increase and later decrease in glomerular filtration rate and filtration fraction. Peak changes in extracellular volume and renal function coincided in time.

Previous evidence indicates that inulin is excluded from the cell and that its space of distribution affords the best method available for the measurement of extracellular volume. Provided that this premise is true, these data suggest that cortisone and ACTH produce a transient but significant shift of water, sodium, and chloride into the measurable extracellular space.

### *The Relation of Mitral Valve Area to Clinical Status in Patients with Mitral Stenosis.* Benjamin M. Lewis, Florence W. Haynes and Ralph J. Spiegl, Boston. Mass. (Introduced by Reginald Fitz).

Thirty patients with mitral stenosis were studied by cardiac catheterization and from these data mitral valve areas and pulmonary arteriolar resistances were calculated by previously published formulae.

From analysis of history, physical examination, electrocardiogram, and x-ray done prior to catheterization, major symptoms and signs were found to begin at a valve area of 1.0 cm.$^2$ (normal 4–6 cm.$^2$). Patients with larger valve areas had few and variable symptoms. Hydraulically, if a normal flow is to be maintained at a stenosis of 1.0 cm.$^2$ or less, left atrial (and therefore pulmonary "capillary") pressure must rise to levels which approach plasma osmotic pressure, with the resultant threat of pulmonary edema. The one compensatory mechanism left such a patient is an increase of pulmonary arteriolar resistance, which, by checking the pumping ability of the right ventricle, would tend to prevent sudden rises in pressure in the capillaries of the lung. Such an increase occurs to a variable degree when the mitral valve area is 1.0 cm.$^2$ or smaller. Thus, as the mitral valve area approaches 1.0 cm.$^2$ in size and before resistance in the pulmonary arterioles rises appreciably, hemoptysis and paroxysmal nocturnal dyspnea are often noted. As the valve narrows farther and the resistance does rise appreciably, cardiac enlargement and signs of right ventricular failure usually follow. Significant linear correlations are found between cardiac enlargement and mitral valve area and between cardiac enlargement and pulmonary arteriolar resistance. Therefore, when the mitral valve area is 1.0 cm.$^2$ or less, the following clinical manifestations become increasingly prominent: disabling exertional dyspnea, edema, hepatomegaly, cardiac enlargement, enlargement of the left auricle and the pulmonary artery, auricular fibrillation and electrocardiographic evidence of right ventricular hypertrophy.

Thus the mitral valve, like most organs of the body, has a reserve capacity to the extent of 80 to 85 per cent before important signs of functional inadequacy appear.

### *The Effect of Cortisone Acetate on Idiopathic Acquired Hemolytic Anemia.* Allyn B. Ley and Frank H. Gardner, Boston, Mass. (Introduced by William B. Castle).

Observations were made in three patients with acquired hemolytic anemia. Each originally presented an enlarged spleen, an anemia with an erythroblastic marrow, reticulocytosis and hyperbilirubinemia, and a positive antiglobulin (Coombs) test. In only one patient were there increases in red cell osmotic and mechanical fragilities. Two of the patients were treated initially with cortisone acetate: in one this was preparatory to splenectomy; in the other the drug has been continued in gradually decreasing dosage. The third patient was first treated with ACTH and then continued on cortisone acetate as maintenance therapy.

In each of the first two patients a significant therapeutic effect was observed. The red cell, hemoglobin and hematocrit values gradually rose and the reticulocyte counts subsided as the serum bilirubin values returned to more normal levels. The Coombs test titers became weaker or negative. In the patient who demonstrated increased red cell fragilities, these abnormalities disappeared. The third patient, whose initial improvement was induced by the use of ACTH has since maintained on cortisone acetate normal blood values and a normal serum bilirubin for 2 months. However, the initial Coombs test titer has shown no change.

It is concluded that cortisone, like ACTH, is an effective agent in the treatment of some patients with acquired hemolytic anemia.

### *Immunization Against Scrub Typhus by Combining Living Vaccine with Chemoprophylaxis.* Herbert L. Ley, Jr. and Joseph E. Smadel,* Washington, D. C.

Earlier studies by Army Research Units in Malaya showed that chloramphenicol given prophylactically suppressed overt scrub typhus in volunteers exposed by sitting in the grass in hyperendemic areas. Typical disease subsequently developed when the last dose was given three weeks following initial exposure but volunteers remained well if prophylaxis was continued for four weeks.

Since inactive scrub typhus vaccines have proved useless in the field, a combined procedure consisting of intradermal inoculation of "attenuated" living *R. tsutsugamushi* followed by intermittent prophylactic administration of 3.0 gram oral doses of chloramphenicol was studied. All eight volunteers receiving vaccine without chemoprophylaxis developed clinical scrub typhus eight to ten days after inoculation. The six given drug on the 8th, 12th and 16th day sickened on the 20th to 23rd day. None of the groups of eight who received their last dose on the 21st, 27th, or 34th day subsequently developed clinical scrub typhus. Eschars appeared at the site of vaccination in all volunteers and rickettsemia occurred in the control and test volunteers.

Intermittent chemoprophylaxis from the 8th to the 21st day following inoculation of rickettsiae suppressed overt disease but permitted a smoldering infection.

### *Quantitative Studies in Man of the Cardiac Effects of Reflex Vagal Stimulation.* Arthur J. Linenthal, Boston, Mass. (Introduced by A. Stone Freedberg).

Certain quantitative aspects of reflex vagal action on the heart, not heretofore described in man, have been studied in order to clarify the role of this reflex in the genesis and treatment of cardiac arrhythmias.

Changes in sino-auricular rate, auriculo-ventricular conduction time and site of impulse formation were determined electrocardiographically following manometrically-controlled carotid sinus pressure in patients with angina pectoris or paroxysmal arrhythmias.

Significantly, this visceral reflex follows well-established principles of neurophysiology applying to various motor and sensory phenomena. Stimulus-response curves for effects on rate and auriculo-ventricular conduction are S-shaped. In the same individual these two cardiac responses, frequently simultaneous, are independent, having different thresholds and different rates of increase and decline. Rapid accommodation occurs if constant pressure is maintained for more than a few seconds. The slowed heart rate rises rapidly, leveling off below the control rate as pressure is continued.

Right and left vagal stimulation may have marked action on both sino-auricular and atrio-ventricular nodes. Varying combinations of the two effects occur in different individuals.

During a two-year period in a patient with angina pectoris, the action on auriculo-ventricular conduction increased markedly, without change in the effect of carotid sinus pressure on the sino-auricular node and the cardiac rate; this was traced to alterations in the heart.

Atrio-ventricular beats may occur during maximum sino-auricular slowing. Increased sino-auricular rate above the control level, supra-ventricular and atrio-ventricular tachycardias and ventricular extra-systoles have been observed after reflex vagal stimulation has been discontinued, during rebound from reciprocal sympathetic inhibition.

Atropine and quinidine may block reflex vagal action on the atrio-ventricular node without altering its effect on the sino-auricular node.

These findings afford a new quantitative approach applicable to the clinical and pharmacological study of this important reflex.

*The Effect of Graded External Pressures on the Vascular Volume of the Leg.* JULIUS LITTER and J. EDWIN WOOD, III, Boston, Mass. (Introduced by Robert W. Wilkins).

Quantitative plethysmographic measurements were made of the vascular volume of the leg, *in vivo*, with the circulation under normal conditions of rest. The blood was first expressed from the leg and thigh by the application of air-pressure (250 mm. Hg) successively to the water-filled plethysmograph on the leg, and to overlapping blood pressure cuffs on the thigh. After the circulation was restored, the increase in leg volume was recorded until reactive hyperemia had subsided and the resting vascular volume was constant. These measurements were repeated over a range of external water pressures on the leg from minus 5 to plus 55 mm. Hg referred to heart level.

A marked decrease in vascular volume of 2.5 per 100 cc. of leg (from 4.0 to 1.5 cc.) was produced by increasing the external water pressure only 10 to 15 mm. Hg. This was attributed to a large decrease in the volume of the veins (and perhaps the venules) due to the reduction of the effective venous pressure to barely greater than zero. When the external water pressure was further increased from 15 to 55 mm. Hg, only a small additional decrease in volume of 0.5 cc. occurred. This was interpreted as being due to decreases in the volume of the capillaries, arterioles and arteries, the venous volume and effective pressure remaining constant.

These results give quantitative information about the volume of different blood vessels *in vivo*, and confirm the *in vitro* studies of other workers showing that the volume of veins depends upon the effective venous pressure, and is independent of absolute pressure. The data also have a practical bearing on the use of elastic compression to accelerate venous flow and prevent thrombo-embolic complications in hospitalized patients.

*Some Studies of Posterior Pituitary and Adrenal Cortical Interrelationships.* C. W. LLOYD, J. F. HARRIS and J. LOBOTSKY, Syracuse, N. Y. (Introduced by R. H. Lyons).

Two normal subjects and five patients with liver disease were injected daily with Pitressin tannote in oil while they were maintained on standardized salt and fluid intakes. Frequent observations of body weight, serum sodium, and urine volume, sodium and corticosteroid, were made. Very little change in any of these measurements occurred in the normal subjects. The patients with liver disease had striking changes, developing hyponatremia and edema as results of the excretion of increased amounts of sodium and the retention of water. Decreases of serum sodium to values as low as 106 m.Eq/l and weight gains as great as 20 pounds occurred in these patients during Pitressin administration. When the serum sodium remained within normal limits, no change in urinary corticosteroid occurred. The urinary corticosteroid rose to high values when hyponatremia had been produced, suggesting the occurrence of an adrenal cortical response. In the presence of sodium depletion and water retention, administration of adrenal cortical extract in addition to the Pitressin caused diuresis and sodium retention.

*Studies on the Metabolism of Cholesterol in Normal Man and in the Nephrotic Syndrome.* IRVING M. LONDON,* GLORIA F. SABELLA and MARTHA M. YAMASAKI, New York, N. Y.

The rate of synthesis of serum cholesterol may be measured by determining the rate at which deuterium is incorporated into the serum cholesterol when an elevated deuterium concentration is maintained at a steady level in the body water. The rise in deuterium concentration in the serum cholesterol represents the appearance in the circulation of newly synthesized cholesterol molecules formed from precursors which have derived their deuterium from the labeled body water. On prolonged maintenance of a steady deuterium concentration in the body water, the isotope concentration in the serum cholesterol rises to a maximum value. The time required to reach 50 per cent of this maximum deuterium concentration is the half life time, $t_{1/2}$, of serum cholesterol, i.e., the time required for half the serum cholesterol molecules present in the circulation to be formed.

A study in a normal adult male revealed a value of about 8 days for the half life time of serum cholesterol. This represents a turnover time, or mean survival time, of about 12 days ($t_{1/2}/\ln 2$).

A similar study has been conducted in a patient with the nephrotic syndrome having marked hypercholesterolemia. The rates of formation of cholesterol of the serum and of the erythrocytes have been determined. The results indicate that the half life time of the serum cholesterol is prolonged. These findings will be discussed in terms of the pathogenesis of the hypercholesterolemia of the nephrotic syndrome.

Studies have been performed on the interrelationships of serum and erythrocyte cholesterol. The incubation of normal mature human erythrocytes with $C^{14}$ labeled acetate reveals that these cells have no significant capacity for cholesterol synthesis. The origin of erythrocyte cholesterol will be discussed in the light of these findings.

### *Entero-Hepatic Circulation of Bromsulphalein: Studies in Man and Dog.* Stanley H. Lorber, Harry Shay, M. J. Oppenheimer and Herman Siplet, Philadelphia, Pennsylvania (Introduced by Richard A. Kern).

Previously demonstrated intestinal absorption of bromsulphalein was further investigated in dogs employing a catheterization technique. Significant serum BSP concentrations in hepatic vein, portal vein and peripheral artery were usually observed within 5 minutes of its intraduodenal instillation; being highest in portal blood and dye appeared in bile within 10–15 minutes. For one minute, following administration of BSP into the portal vein, dye concentrations in the hepatic vein exceeded those in peripheral artery. From 2 to 15 minutes, BSP concentrations in peripheral artery exceeded those in hepatic vein but at 30 and 45 minutes little difference between the two was observed.

To correlate these observations with the clinical BSP test, cholecystectomized patients were administered the 2 and 5 mg./kg. doses intravenously with and without common duct aspiration. Significant correlation between dye removal and change in serum BSP levels was observed only after the 5 mg./kg. dose. Marked decreases in serum BSP concentrations occurred when a significant percentage of the intravenously administered dye was recovered in the bile. The effect of reabsorbed dye upon serum levels was more readily evident after the intravenous administration of BSP than after its intestinal instillation. This may be due to "saturation" of the excretory mechanism under the former conditions.

We conclude from these data that the influence of an enterohepatic circulation upon the BSP test is a quantitative one and suggests that the smaller dose is preferable for clinical use. The relationship of these observations to EHBF will be discussed.

### *The Syndrome of Short P–R Interval, Normal QRS Complex and Paroxysmal Rapid Heart Action.* Bernard Lown and Frank Ganong, Boston, Mass. (Introduced by Samuel A. Levine).

Although the Wolff-Parkinson-White syndrome has been widely reported, the association of paroxysmal tachycardia and a short P–R interval without QRS abnormality has not been recognized.

From the electrocardiographic files of the Peter Bent Brigham Hospital two hundred cases with a P–R interval of 0.12 or less were compared with a control group of 200 cases with P–R intervals of 0.16 and 0.18 seconds. Cases on digitalis or with conditions known to predispose to paroxysmal tachycardia were excluded from both groups.

Among the 200 cases with the short P–R intervals, there were 16 cases with anomalous A–V conduction. Four of these had paroxysms of rapid heart action. Of the remaining 184 cases with normal QRS complexes, 19 had paroxysmal tachycardia. In the 200 control group there was only one such case with tachycardia. Thus there is a markedly greater incidence of rapid heart action in the short P–R group.

These cases differ from the Wolff-Parkinson-White syndrome in several ways. Their usual P–R interval is 0.12 second, rarely less, the QRS duration is less than 0.09 and the P–J interval is seldom more than 0.20 second. Slurring of the initial portion of the R wave is absent. Unlike the Wolff-Parkinson-White they do not exhibit "normalization" of the P–R and QRS even after many years follow-up.

In this study it was noted that cases of Addison's disease have P–R intervals longer than 0.15, while cases with Cushing's syndrome have P–R intervals less than 0.14 second; furthermore massive doses of cortisone seem to shorten the P–R interval. Whether endocrine factors are operative in the genesis of this entity is as yet uncertain.

These cases constitute an easily recognizable clinical group consisting of paroxysmal tachycardia, preeminently in females, starting in middle life, with a snapping mitral first sound, who have a constantly short P–R and a QRS devoid of slurring or prolongation.

### *A Comparison of the Effects of ACTH and Cortisone in Nephrosis.* John A. Luetscher, Jr.,* Quentin B. Deming and Ben B. Johnson, San Francisco, Calif.

Twenty-nine patients with nephrosis, with or without clear evidence of glomerulonephritis, have received cortisone or ACTH for 5 to 16 days for a total dose of 500 to 2100 mg. Of 18 courses of treatment with cortisone, 10 resulted in complete elimination of edema, 3 led to partial diuresis, and 5 cases had no beneficial result. Of 14 cases treated with ACTH, 10 lost all edema, while 3 showed no significant diuresis. Treatment with either agent usually increases the clearances of creatinine or inulin and causes retention of sodium and water during the initial days of treatment, followed by release on later days. After treatment, there is usually diuresis and more or less reduction of proteinuria, with increased plasma protein and albumin levels. ACTH may initiate an impressive elimination of water and sodium during the latter days of treatment, and proteinuria may decline at this time: such changes are rarely seen until after discontinuance of cortisone treatment. Reduction in serum cholesterol usually follows ACTH treatment. A reduction in the abnormal sodium-retaining activity of urinary corticoids is observed whether diuresis occurs during or after treatment, but fails to occur when edema is unaffected. Improvement in urinary sediment is often observed when a reduction in proteinuria occurs.

The duration of a remission following ACTH or cortisone varies greatly. It may be interrupted by an intercurrent infection or there may be a gradual return of proteinuria or edema without obvious cause. Sustained improvement lasting many months is possible.

*Protein Studies on Treponemal Immobilizing Antibodies.* HAROLD J. MAGNUSON,* HENRY TAUBER, CHARLOTTE McLEOD and WARFIELD GARSON, Chapel Hill, N. C.

Cohn Method X for the fractionation of normal human plasma has been adapted to the fractionation of plasmas or serums from approximately 50 patients with untreated early syphilis in whom treponemal immobilizing antibodies could be demonstrated. The chemically separated fractions were further studied by electrophoresis. These studies indicate that the immobilizing substances are present in Precipitate I + III − 1,2.3 associated with the high molecular weight isoagglutinins and a small amount of prothrombic protein; and in Precipitate I + III − 3 associated with the high molecular weight antihemophilic globulin, cold insoluble globulin, and a small amount of plasminogen. These precipitates which contain nearly all of the spirocheticidal substances represent a small percentage of the blood proteins. Unsuccessful attempts have been made to demonstrate binding of these spirocheticidal substances to pathogenic *T. pallidum* through the use of $I^{131}$ tagged and fluorescein tagged serums and serum fractions.

*The Effect of a Series of Bacteria and Their Products on the Human Leukocyte.* SAMUEL P. MARTIN, IVAN L. BENNETT and S. CHAUDHURI, Durham, N. C. (Introduced by David T. Smith).

The effect on human leukocytes of a series of bacteria and their carbohydrate products has been tested. Morgan has noted that leukocytes from splenic implants of guinea pigs did not migrate toward the carbohydrate extracted from S. typhosa. With the same carbohydrate, Delauncy noted marked inhibition of diapedesis of leukocytes, and Miles and Nivens explained the paucity of leukocytes in injured tissue on the inability of the animal to maintain adequate blood pressure. In our studies leukocytes were observed by a slide cell technique after in vitro and in vivo administration of various bacterial products.

With the use of the intact organism in vitro, S. typhosa and Ps. aeruginosa inhibited the migration of leukocytes in concentration of $6 \times 10^6$ organisms/ml (approximately one per leukocyte). M. aureus and D. pneumoniae had no inhibitory effect in concentrations of $6 \times 10^8$ organisms/ml. The purified carbohydrate of S. typhosa (Morgan), P. aeruginosa (Baxter), and S. Marcesens (Shear), as well as filtrates, inhibited the migration of leukocytes. As the dosage was decreased, stimulation was noted. Polysaccharides from D. pneumoniae type III showed no inhibition.

In the study of blood drawn before and after the administration of heat killed S. typhosa, a moderate stimulation of leukocytes is noted during the first two hours after the material is given. This stimulation is prevented by the administration of ACTH.

It would appear that the alteration of migration of leukocytes may reflect a change in the cell and that this may be important in the distribution of leukocytes following the administration of these compounds.

*Thyroid Hyperplasia Disproportionate to Function.* GEORGE B. McADAMS, New Haven, Conn. (Introduced by William T. Salter).

In distinguishing between toxic and non-toxic goiter, a single determination over the gland of radioactive pick-up (due to $I^{131}$) may be misleading. Such a measurement is more likely to indicate thyroid hyperplasia than thyroid hyperfunction. If repeated determinations are made with the Geiger-Mueller counter (following the test dose of $I^{131}$), however, it is possible to estimate the rate at which iodide is transformed into thyroxine and secreted. This TMR (thyroid metabolic rate) correlates well with the concentration of serum thyroxine or "hormonal" iodine. In smouldering or burned-out Graves' disease (*forme fruste*), the TMR usually lies within normal limits; whereas the maximal uptake of radioactivity in the gland is extremely high (due to hyperplasia). The TMR normally is between 0.18 and 0.32 mg. per day *per square meter* of *body surface*, in terms of L-thyroxine. In myxedema the TMR approaches zero; and in brisk hyperthyroidism it exceeds half a milligram of L-thyroxine per day per square meter. Evidently the best way to determine thyroid function is to estimate the amount of L-thyroxine produced per day. One should not be misled by the size or mass of thyroid parenchyma.

*The Action of Protoveratrine on Renal Function in Hypertension.* E. MEILMAN, Boston, Mass. (Introduced by S. L. Gargill).

The ability of protoveratrine to lower blood pressure in a variety of conditions characterized by hypertension affords the opportunity of studying such patients during periods of elevated blood pressure and lowered or normal blood pressure. Since this fall in blood pressure is the result of a decreased peripheral resistance, it was important to determine kidney function during this period.

Renal clearance studies (inulin and PAH) have been carried out 40 times in 35 patients with hypertension of varied etiology before and after protoveratrine. In most of these, clearance materials were administered with a constant speed infusion pump. In ten studies after single i.v. injections of protoveratrine, a moderate to marked fall in GFR and a lesser fall in ERPF usually occurred during the most marked blood pressure fall; both functions recovered toward but not above control levels as the blood pressure rose to former levels. Since such measurements were made during a constantly changing blood pressure level, further studies during a continuous i.v. infusion of protoveratrine were made while the blood pressure was being maintained at a steady low level for 2–4 hours.

These studies demonstrate that GFR usually approaches or reaches control levels 1–2 hours after the first lowering of blood pressure, while ERPF reaches or exceeds control values in this time. These observations are consistent with the hypothesis that the kidney in essential hypertension as well as chronic renal disease is capable of undergoing vasodilatation following the administration of protoveratrine.

The vasodilatation is accompanied by a marked decrease in the resistance of the kidney.

### *The Causes of Constriction of the Digital Vascular Bed.* Milton Mendlowitz,* New York, N. Y.

In the digital circulation the sympathetic nervous component of vasoconstriction can be eliminated by the application of heat indirectly supplemented by the intravenous administration of TEAC. When this is done in hypertensive patients, residual vasoconstriction, the mechanisms for which remain obscure, can often be revealed.

The possibility that metabolic factors are responsible for this residual vasoconstriction was investigated by studying oxygenation and hydration. In severe anemia, when the slight decrease in viscosity was incorporated in the calculations, there was no evidence of intrinsic vasodilatation. In clubbed fingers, moreover, and in coarctation of the aorta, intrinsic vasoconstriction was not a response to increased perfusion pressure in the digital arteries.

Hydration was studied by comparing the circulation in contralateral digits in patients with unilateral lymphedema, usually following radical mastectomy. No significant differences were found beyond those in a control group. In one patient with malignant hypertension, studied repeatedly, salt depletion produced a gradual decrease in non-neurogenic or intrinsic digital vascular resistance. The factors of chemical composition of intra- and extra-cellular fluids were not concomitantly studied.

In every patient subjected to dorso-lumbar sympathectomy some return of neurogenic vasoconstriction in the toe could be demonstrated. In addition, in at least one-third of these patients, an increase in non-neurogenic intrinsic vascular resistance followed sympathectomy. Infusion of benzodioxane after prior release of residual sympathetic nerve tone by indirect heat and TEAC failed to demonstrate any relationship between this intrinsic narrowing and circulating epinephrin. The available evidence suggests disuse atrophy and consequent shortening of arteriolar circular muscle as the probable cause of this decrease in vascular caliber.

### *Observations on the Effect of Intravenous Human Serum Albumin in Hypersensitivity Reactions and Various Arthritic States.* William R. Merchant, Thomas McP. Brown,* Lucille B. Robinson and Ruth H. Wichelhausen, Washington, D. C.

The association of serum protein alterations with various disease states is being recognized more frequently with the increased application of electrophoresis and other techniques. In those diseases with an infectious etiology or a possible hypersensitivity mechanism, attention has usually been directed toward abnormalities in the globulin fractions. The significance of the relation of the albumin to the globulin fractions has been obscure.

Relatively minute amounts of human serum albumin interfere with the zinc turbidimetric measurement of gamma globulin. In patients with rheumatoid arthritis human serum albumin given intravenously produced temporary variations of clinical effect from marked symptomatic improvement to mild exacerbation of symptoms. The latter was usually associated with larger quantities of albumin. A transient rise in albumin and depression of gamma globulin fractions, as determined electrophoretically, was noted following single injections of albumin. These changes paralleled those noted in the sera of patients receiving cortisone.

To correlate the comparative clinical and serum protein effects further, known sensitivity reactions were studied. In three patients with serum sickness and in seven patients with reactions due to penicillin, prompt relief of their symptoms was obtained when small quantities of albumin were administered intravenously. One patient with aplastic anemia had four successive transfusion reactions. Three subsequent transfusions were given without reaction when administered concomitantly with albumin. Tuberculin hypersensitivity and the precipitin reaction in serum sickness could also be modified by human serum albumin.

Three patients with gout who were completely refractory to colchicine were studied. Albumin alone produced no effect in these patients. However, when albumin administration was immediately followed with colchicine marked clinical improvement was obtained.

These observations would suggest that serum albumin independently, or in its interrelationship with globulin, may modify the manifestations of known hypersensitivity and various arthritic states.

### *Observations on the Pressor Response Occurring During Dialysis with the Artificial Kidney.* John P. Merrill, Walter T. Goodale and Roy C. Swan, Boston, Mass. (Introduced by Henry A. Christian).

The pressor response accompanying dialysis with the artificial kidney is characterized by a rise in both systolic and diastolic pressure with a slight increase in pulse pressure and pulse rate. The etiology of this response is at present not clear but conclusions as to the dynamics of the response and its clinical significance can now be made. The possible contribution of the glucose content of the bath, of changes in plasma volume, in extracellular fluid, and the mechanical effect of transport of blood through the apparatus have been eliminated by controlled experiments. An increase in cardiac output, as measured by the technique of cardiac catheterization, has been implicated in the majority of these responses. Certain hypertensive patients may show no increase in cardiac output, but a marked increase in peripheral resistance and mean arterial blood pressure. One such patient, however, given the peripheral vasodilator, protoveratrine, at the height of the pressor response maintained her mean arterial blood pressure by doubling the cardiac output in the face of a halved peripheral resistance. In all cases where measured, pulmonary artery pressures were unchanged.

Two patients with hypotension refractory to infusion of large amounts of plasma and blood have been studied. In neither of these patients was there evidence of peripheral vasoconstriction. Both patients were jaundiced and anuric. Initial cardiac output was low, and in one patient the pressor response occurring during dialysis was accompanied by a 35 per cent increase in cardiac output and resumption of urine flow. In one nephrotic patient, without nitrogen retention, dialysis was accompanied by a marked increase in glomerular filtration rate from 75 cc/minute to 170 cc/minute and a marked increase in urine flow and sodium excretion. A second patient with long-standing Laennec's cirrhosis and edema showed a marked increase in cardiac output with a similar increase in urine flow and sodium excretion.

*The Effects of Experimental Pericardial Tamponade on Pressures in the Pulmonary Circulation.* JAMES METCALFE and JOHN W. WOODBURY, Boston, Mass. (Introduced by C. Sidney Burwell).

Peripheral venous, peripheral arterial, pulmonary venous (in four cases "pulmonary capillary") and pulmonary arterial pressures have been measured in a series of dogs subjected to progressive pericardial tamponade by a method permitting normal respiration with a closed thorax.

The well documented rise in peripheral venous pressure and fall in peripheral arterial pressure is again confirmed. With increasing intrapericardial pressure, pulmonary venous pressure (or "pulmonary capillary") is found to rise in every case. In spite of this consistent rise in pulmonary venous pressure, the filling gradient of the left ventricle (pulmonary venous pressure minus intrapericardial pressure) gradually becomes zero. At this point in the procedure peripheral arterial pressure falls precipitously. Depending on its resting level, mean pulmonary arterial pressure rises or falls to approximate that level of pulmonary venous pressure which is reached at the height of tamponade.

These experiments, therefore, for the first time make it clear that in tamponade there is congestion of the pulmonary vascular system as well as of the systemic vascular system. The significance of these observations will be discussed.

*Observations on Renal Mechanisms for Sodium and Potassium Excretion in ACTH-induced Diuresis of Nephrotic Edema.* JACK METCOFF, C. P. RANCE and N. NAKASONE, Boston, Mass. (Introduced by C. A. Janeway).

The renal capacity to excrete osmotic loads delimits some aspects of tubular function. Insulin and para-aminohippurate clearances with coincident loads of sodium salts of the non-reabsorbable anions $S_2O_3^{=}$ and PAH$^-$ were obtained initially in three edematous nephrotic children and subsequently before, during, and after ACTH-induced diuresis. Comparable load studies were done in normal untreated children.

Relative osmotic diuresis occurred in nephrotics despite edema or ACTH therapy. Patterns of fixed-cation excretion varied. Sodium plus potassium excretion usually exceeded that of non-reabsorbable anion. Cation excess was largely balanced by diminished chloride reabsorption.

The aliquot of infused sodium contributing to the tubular load (fractional filtered load) was estimated from retention, concentration and apparent volume of distribution. Normal children excreted the total fractional filtered load plus additional quantities of filtered sodium to satisfy obligatory anion demands. Edematous nephrotic children reabsorbed 50–90 per cent of the fractional filtered sodium load. This reabsorption appeared asymptotic. ACTH-induced diuresis began during therapy. Coincidentally, and in the non-edematous state thereafter, reabsorption of the sodium load was markedly curtailed.

Osmotic diuresis of potassium occurred with $Na_2S_2O_3$ and NaPAH loads during edema. Potassium excretion exceeded amounts simultaneously filtered in over seventy periods. During and after ACTH-induced diuresis, potassium excretion was largely independent of urine flow, simulating the normal load response.

These observations suggest that during nephrotic edema, renal tubular cells excessively but not maximally reabsorb sodium. Further reabsorption of a superimposed sodium load occurred during ACTH therapy, unless diuresis of edema fluid had begun. With diuresis, sodium reabsorption was markedly reduced. Potassium excretion, relatively unaltered by ACTH therapy, conformed with the hypothesis of tubular cation exchange. Potassium secretion suggested a more distally located mechanism for rapid mobilization of cation.

ACTH-induced diuresis may be mediated by relative sodium rejection and concomitant restoration of normal potassium transfer by renal tubular cells.

*Relationship of Allantoin and Endogenous Creatinine Clearances to Glomerular Filtration Rate in Man.* BENJAMIN F. MILLER,* ALEXANDER LEAF, AUDLEY R. MAMBY and ZELMA MILLER, Boston, Mass.

The allantoin, the endogenous creatinine chromogen, and the specific creatinine clearances have been proposed for the determination of the glomerular filtration rate to obviate the intravenous infusion required by the inulin clearance. In this study we have compared these three clearances with the inulin clearance to assay their reliability for filtration measurements.

In seven patients with a variety of renal lesions, clearance ratios were determined in a total of twenty-two periods. The allantoin clearance equalled the inulin clearance in seven periods, and was lower in the other fifteen. In eight comparisons, the ratio of allantoin to inulin clearance fell below 0.8. Ratios of 0.73, 0.75, and 0.64 were observed in a patient with severe cardiac failure.

The clearances of *creatinine chromogen* ("apparent creatinine") and of *specific creatinine* did not prove acceptable as true measures of filtration rate. *Creatinine chromogen*/inulin ratios as high as 1.64 were obtained in a patient suffering from long-standing glomerular nephritis, and as low as 0.56 in the cardiac patient mentioned above. The highest *specific creatinine*/inulin clearance ratio, 1.74, was obtained in the patient with the lowest renal function (average inulin clearance of ten cc.). The lowest ratio, 0.98, was found in the patient with severe cardiac failure. The endogenous *creatinine chromogen* was determined by the Bonsnes and Taussky modification of the Jaffe reaction. *Specific creatinine* was measured by the Miller-Dubos enzymatic method.

The variations in clearance ratios of allantoin/inulin and endogenous creatinine/inulin are interesting because they may eventually lead to a better understanding of tubular function in various renal syndromes. We feel, however, that the variations are so large that the allantoin and creatinine clearances should be considered unreliable measures of filtration rates in disease.

### *Erythrocyte and Serum Cholinesterase in Ulcerative Colitis.* Hugo C. Moeller, J. Alfred Rider and Joseph B. Kirsner,* Chicago, Ill.

Hyperactivity of the parasympathetic nervous system with resultant hypermotility of the bowel has been implicated in the pathogenesis of ulcerative colitis. The neurogenic hyperfunction may be accompanied with an increased concentration of acetylcholine, and, correspondingly, cholinesterase, at the myoneural junction. Whereas serum cholinesterase parallels the nutrition of the individual, erythrocyte cholinesterase may reflect cholinesterase at the myoneural junction.

Cholinesterase activity was measured by the Warburg manometric technique in 50 normal individuals and in 35 patients with ulcerative colitis; results are expressed as microliters of $CO_2$ liberated during an average 5-minute period by 0.1 cc. of serum or erythrocytes. In the normal group, erythrocyte cholinesterase varied from 71 to 102, averaging 86; serum cholinesterase ranged from 27 to 46, averaging 36. In active ulcerative colitis erythrocyte cholinesterase tended to be higher, ranging from 79 to 137, averaging 99; serum cholinesterase was lower than normal, varying from 11 to 36, averaging 23. Erythrocyte cholinesterase was normal in patients with myasthenia gravis, gastric carcinoma and polycythemia vera. However, high values were observed also in peptic ulcer and pernicious anemia, indicating that the increased erythrocyte cholinesterase in ulcerative colitis is not specific for this disease.

Twenty-four patients with moderate to severe colitis received ACTH or cortisone; the clinical response was good in most cases. During the active stage, erythrocyte cholinesterase ranged from 81 to 137, averaging 108; serum cholinesterase varied from 8 to 34; averaging 22. At the time of greatest improvement during ACTH therapy, erythrocyte cholinesterase ranged from 72 to 104, with a mean of 85; serum cholinesterase varied from 17 to 44, averaging 27. Fluctuations in erythrocyte cholinesterase paralleled the recurrences and remissions of ulcerative colitis.

### *Studies on in vitro Potassium Accumulation.* Gilbert H. Mudge, New York, N. Y. (Introduced by John V. Taggart).

Thin slices of rabbit kidney cortex lose K during preparation in chilled isotonic NaCl and reaccumulate K from a low external concentration (10 mEq./l.) during incubation under optimal conditions. The process of reaccumulation was studied with simultaneous measurements of tissue respiration and electrolyte composition. K accumulates as a net exchange for Na. Depression of aerobic oxidation by drug action, changes in oxygen tension, temperature, etc., produces a simultaneous depression in K uptake.

All metabolic inhibitors which depress respiration likewise depress the accumulation of K. Anoxic anoxia, cyanide and arsenite depress these functions in a parallel manner. A larger group of compounds (including azide, mercuric chloride, organic mercurials and Benemid) have a marked effect on electrolyte metabolism at concentrations significantly lower than those required to depress oxygen consumption. Studies of 2,4-dinitrophenol and related compounds showed a good correlation between depression of K uptake and inhibition of the generation of high energy phosphate bonds. Inhibitors of carbonic anhydrase, cholinesterase and alkaline phosphatase had no effect on K accumulation.

Tissue hydration was slightly increased by anoxic anoxia but more so by specific inhibitors. Tissue osmotic pressure (measured as the sum of Na plus K) remained essentially constant, regardless of changes in water content, in every experiment with a constant external osmotic pressure. Depression of metabolic activity is, therefore, associated with an isosmotic increase in tissue hydration. No evidence was found to support the concept that the cells are normally hypertonic to their environment.

K accumulation is regarded as an example of active transport. Conditions affecting net accumulation are now being examined with the use of radioactive K.

### *An Estimation of Portal Venous Pressure by Occlusive Catheterization of an Hepatic Venule.* J. D. Myers* and W. Jape Taylor, Durham, N. C.

Knowledge of portal venous pressure in man is confined largely to measurements at laparotomy. A ready estimate of portal venous pressure in intact man would be valuable in diagnosis and in correct assessment of the therapeutic procedures such as porto-caval shunts. Dexter's experience that pulmonary "capillary" pressure is reflective of pulmonary venous pressure suggested similar exploration of the more complex hepatic circulation.

In 18 observations on 10 cats, selected because of similarity of the feline and human hepatic circulations, pressures, measured through an intravenous catheter passed occlusively into a distal hepatic venule, ranged from 6 to 13.5 mm. Hg (mean 8.6). Almost simultaneous direct determinations of portal venous pressure agreed closely (mean difference 0.6 mm. Hg; range −3.5 to +2.5). Epinephrine raised both pressures to a similar degree. Ligation of the hepatic artery caused no significant reduction in either pressure.

In 12 humans without liver disease, the mean pressure was 4.8 mm. Hg in an hepatic venule occluded by a catheter passed via an antecubital vein. The free pressure in a large central hepatic vein averaged 3.3 mm. Hg, there being no significant gradient between the two pressures. Blood withdrawn from occluded hepatic venules had essentialy identical glucose and oxygen contents with hepatic venous blood indicating no massive circulatory obstruction from the catheterization.

Seven patients with portal hypertension from Laennec's cirrhosis gave pressures in the occluded venule from 11.5 to 32 mm. Hg (mean 20.1). These pressures agree in magnitude with portal venous pressures recorded by others at laparotomy in portal hypertension. The pressures between the control and hypertensive groups did not overlap. The pressure gradient from the occluded venule to a central hepatic vein in the cirrhotics averaged 14.4 mm. Hg in contrast to the small normal gradient.

These preliminary studies suggest that the pressure recorded from an occluded hepatic venule is indicative of portal venous pressure and can be used to estimate portal hypertension in man.

### *The Crystallization of "Direct" and "Indirect" Bilirubin from Human Serum and Their Respective Properties.* VICTOR A. NAJJAR* and BARTON CHILDS, Baltimore, Md.

Serum (0.1 − 1.0 ml.) from patients with jaundice is brought to pH 6.0 extracted into ether and from ether into pyrophosphate buffer pH 9.0. The pH is then lowered to 7.4 and cooled to 4°C. Crystals from "indirect" serum form readily and are long thin needles with occasional plates, whereas, crystals from "direct" serum are slow forming and are short blunt rods. The extracted bilirubins retain their respective "direct" and "indirect" diazo characteristics, are free of protein, dialyzable, and have an absorption maximum at 440 mμ in pyrophosphate buffer pH 8.2. Under appropriate conditions they can recombine with normal non-jaundice serum, retain unaltered diazo characteristics, become non-dialyzable at pH 7–8.2, and the absorption maximum shifts back to 470 mμ where it was in the original jaundice serum. "Indirect" bilirubin combines equally well with albumen and γ globulin on a molar basis. It becomes "direct" reacting after mild alkali treatment pH 10.5 with a release of acidic groups. This reaction is not reversible. Upon fractionation with ammonium sulfate the original "indirect" jaundice serum and the "indirect" pigment combined with normal serum behave identically. Bilirubin begins to come down with the proteins at 0.28, reaches a maximum at 0.40 and is completely precipitated at 0.48 saturation. Similarly, "direct" jaundice serum and "direct" bilirubin recombined with normal serum behave the same. The "direct" bilirubin, however, begins to come down at 0.38, reaches a maximum at 0.44, and precipitates completely at 0.56 saturation.

### *The Effects of Pitressin on the Excretion of Water and Electrolytes in Normal Subjects and Patients with Cirrhosis of the Liver and Ascites.* WILLIAM P. NELSON, III, and LOUIS G. WELT, New Haven, Conn. (Introduced by David M. Kydd).

Normal subjects and patients with cirrhosis of the liver and ascites have been compared with respect to the intensity and duration of their response to pitressin. An infusion of 5 per cent glucose in water was administered so as to attain and maintain a positive balance of one liter of water throughout the entire study. This promotes a relatively steady maximal rate of excretion of water, due, presumably, to a continuous suppression of secretion from the posterior pituitary gland. Two and one-half or one hundred milliunits of pitressin were then administered intravenously, which was followed by a sharp antidiuresis. The rates of excretion of electrolytes and water were observed until the latter returned to the original maximal level.

There was no difference between the normal and the cirrhotic groups in the time required to attain a maximal rate of excretion of water from the original hydropenic state although these rates were strikingly lower in certain of the cirrhotic patients. The per cent decrease in the rate of excretion of water was greater with the larger dose of pitressin but did not differ in the two groups. In addition, the time required for recovery from the antidiuresis was the same in the normal subjects and patients with cirrhosis. In all except one instance the rates of excretion of sodium and chloride decreased.

There is no evidence of a delayed inactivation or excretion of endogenous antidiuretic hormone in patients with cirrhosis of the liver as compared with normal subjects, nor is there evidence of increased sensitivity, delayed inactivation or excretion of administered pitressin in these doses. Pitressin did not promote an increased rate of excretion of sodium or chloride.

### *Changes in Plasma and Erythrocyte Sodium, Potassium, and Water During Recovery from Diabetic Coma.* GEORGE NICHOLS, JR. and NANCY NICHOLS, Boston, Mass. (Introduced by Alexander Marble).

Changes in plasma and erythrocyte sodium, potassium, and water in ten cases of recovery from diabetic coma were studied. No patients had evidence of intrinsic renal damage.

Values obtained were compared with those found by the authors in thirty-seven normals. Total plasma and red cell volumes were calculated by a new method presented by the authors.

On admission, plasma was depleted of sodium and water and total potassium. Erythrocytes showed normal water, low sodium and potassium.

During the early stages of treatment, where large amounts of 0.85% sodium chloride and insulin were given, plasma water and sodium were restored rapidly to normal values. Plasma potassium dropped precipitously in concentration and also showed a drop in total quantity.

The red cells became dehydrated and showed a loss in total potassium during this period.

In the latter stages of recovery, when patients had resumed normal, oral diets, erythrocyte water and potassium slowly returned to normal, often requiring eight to ten days. Plasma potassium reached normal concentrations in four to ten days.

Red cell sodium values were slightly low on admission, fell during therapy, and returned towards normal with the potassium. In some cases, this return was slower than that of potassium.

No correlation between these electrolyte and water changes and the level of the blood sugar, either at the outset or during therapy, was apparent.

*Effect of Sodium Depletion and Repletion on Renal Function and Body Fluids During Uremia.* J. F. NICKEL, P. LOWRANCE and E. LEIFER, New York, N. Y. (Introduced by Joseph C. Turner).

"Azotémie par manque du sel" is generally attributed to diminished glomerular filtration secondary to a change in body fluid volume during salt deprivation. Studies of the effect of sodium depletion in four patients with renal insufficiency due to malignant nephrosclerosis, chronic diffuse glomerulonephritis and polycystic disease have revealed that hypofiltration may develop under these conditions without change in arterial pressure or in composition and volume of body fluids. Following control determinations, rapid depletion of body sodium was achieved by salt-poor diet and ingestion of a cationic exchange resin (Lilly), 45 to 60 Grams per day for six days. After this period, filtration (inulin clearance) had fallen by 45 per cent, on the average, although it did not change in one normal subject. P-aminohippurate clearances fell to approximately the same extent in all, presumably indicating intra-renal vasoconstriction. The blood urea nitrogen rose sharply in two during sodium withdrawal. Sodium and chloride output diminished markedly but not to the same extent as in the normal. Potassium excretion increased somewhat in the patients with renal insufficiency but fell in the normal. Despite these changes serum electrolyte concentrations were not significantly affected and there was no appreciable alteration in total body water (antipyrine), extracellular water (inulin space), and plasma volume (T-1824). Maximal PAH excretion diminished in every instance, roughly to the same extent as electrolyte excretion. PAH loading resulted in augmented output of sodium; the urinary hydrogen ion concentration rose in the normal under these conditions but not in the others. In the latter, sodium depletion appeared to exaggerate potassium output during PAH loading so that potassium clearances exceeded inulin clearances. These alterations appeared to be reversible and cleared on repletion with sodium.

*Clinical Evaluation of Various Types of Ballistocardiographs.* JOHN L. NICKERSON, JAMES A. L. MATHERS and DOUGLAS S. SJOBERG, New York, N. Y. (Introduced by Robert L. Levy).

In view of the differing physical characteristics of the numerous ballistocardiographs in current clinical use a series of observations has been made on a group of normal individuals and a group of patients with cardiovascular disorders using representative instruments of the various types. In the normal group, pattern similarity with the various instruments is more frequent than in the cardiovascular group where one may, with certain instruments, fail to detect obvious cardiovascular abnormalities. Observations made on patients in whom the clinical status was improved by surgical or drug therapy have also shown the inability of certain instruments to detect changes towards normal. In an attempt to explain the divergences between the patterns obtained with the various ballistocardiographs, theoretical and experimental studies have been made and will be discussed.

*Postural Changes in the Cerebral Circulation, Studied by Continuous Oximetric and Pressure-Recording Techniques.* JOHN L. PATTERSON, JR., and JOHN L. CANNON, Atlanta, Ga. (Introduced by A. J. Merrill).

Under conditions in which the oxygen uptake of the brain remains essentially constant, the reciprocal of the cerebral arteriovenous oxygen difference, $1/(A\text{-}V)_{O_2}$, will vary directly with the cerebral blood flow (CBF). Advantage can be taken of this relationship to measure changes in CBF continuously. Studies with the nitrous oxide technique have shown that the mean cerebral oxygen consumption during 65° head-up tilting does not change in non-fainting subjects.

In the present studies continuous measurements of cerebral $(A\text{-}V)_{O_2}$ difference were made with two absolute-reading oximeters. Per cent arterial oxyhemoglobin saturation was obtained with an earpiece, and internal jugular venous saturation with a cuvette. Blood was withdrawn at constant slow rate through the cuvette with a syringe-pump. Arterial and jugular venous pressures were recorded from strain gauges mounted at ear level.

In ten non-fainting subjects, tilted head-up to 65°, the fall in CBF, indicated by changes in $1/(A\text{-}V)_{O_2}$, averaged 20 per cent, but varied from 0 to 40 per cent. In four other subjects, smaller angles of tilt produced smaller changes in CBF.

The CBF began to fall within one minute after tilt and on return to recumbency promptly rose to or above base-line levels. Jugular venous and arterial pressure changes showed directional correlation. In most subjects jugular pressure fell to subatmospheric levels after tilt, contributing materially to the net cerebral perfusion pressure (NCPP = arterial-internal jugular venous pressure).

Both in postural hypotension and vasodepressor syncope (four subjects) fainting occurred when the NCPP approached 15 mm. Hg. The NCPP and $1/(A\text{-}V)_{O_2}$ correlated closely until syncope became imminent. In a postural hypotensive the NCPP and CBF progressively fell until the faint occurred, whereas in normals both functions were readjusted at a lower level after tilt and later fell precipitously just before the faint.

### *The Cerebral Circulation and Metabolism in Chronic Pulmonary Emphysema with Observations on the Effects of Inhalation of Oxygen.*

John L. Patterson, Jr., Albert Heyman * and T. Whatley Duke, Atlanta, Ga.

Reduction in arterial oxygen tension or elevation of carbon dioxide tension have dilator effects upon cerebral blood vessels. Similar changes in blood gas tensions often develop in chronic pulmonary emphysema. The administration of oxygen to such patients alleviates the anoxemia but may result in untoward reactions, such as drowsiness and convulsive seizures.

The cerebral blood flow (CBF) and oxygen consumption ($CMRO_2$) were determined by the nitrous oxide technique before and during the administration of oxygen in nine patients with chronic pulmonary emphysema, seven of whom had cor pulmonale, and in eight control subjects.

The CBF in the patients with emphysema was elevated to a mean value of 68 cc., compared with 50 cc./100 Gm. brain/min. in the control subjects, while the $CMRO_2$ was slightly below the control value. There was a tendency for patients with the lowest values for arterial $pO_2$ and the highest values for $pCO_2$ to show the highest blood flow values, and the converse was also true. During inhalation of 85–100 per cent oxygen, the mean CBF in the patients with emphysema rose to 79 cc., but in contrast, fell by 12 per cent in the control subjects. The maximum CBF attained during oxygen inhalation was 113 cc./100 Gm./min. Two patients who had the lowest flow values while breathing air showed a decrease in CBF with oxygen. The mean $CMRO_2$ did not change and mental aberrations did not develop during the 30 minutes of oxygen administration.

The increased CBF found in the emphysematous patients breathing air was probably a reflection of the high mean arterial $pCO_2$ of 58 mm. Hg and low $pO_2$ of 39 mm. Hg. Oxygen inhalation, by depressing pulmonary ventilation, caused a further increase (12 mm. Hg) in $pCO_2$. This increase in $pCO_2$ apparently more than offset the much larger rise in $pO_2$, and thus produced an increase in cerebral blood flow.

### *Comparative Studies of Cortisone Administered Orally and Intramuscularly to Patients with Rheumatoid Arthritis.*

M. Patterson, C. H. Adams and C. Stevenson, New York, N. Y. (Introduced by Richard H. Freyberg).

During one year, in approximately sixty patients with rheumatoid arthritis, the effects of cortisone administered orally have been compared to effects of this hormone injected intramuscularly. Usually cortisone was given by both routes at different times in the same patients.

Most effects were strikingly similar whether the hormone was given orally or parenterally. There were some significant differences. Cortisone is absorbed more quickly from the gut than from the muscles, as shown by a quicker antirheumatic effect and an earlier reduction in circulating eosinophiles. The same single dose of hormone produced quicker antirheumatic effects when given orally, but the effects disappeared more quickly than when it was given intramuscularly.

Many schedules of administration were studied and compared. Usually the most effective manner of administration of cortisone orally was in three doses at approximately six hour intervals, beginning on arising, with one third to one half of the daily amount given in the first dose, and one third or one fourth given in the second and third doses. When so administered, equivalent clinical effects were produced orally by 1.2 times the daily intramuscular dose—a surprisingly low ratio. No important undesirable effects resulted from oral administration. Cirrhosis of the liver, gastric anacidity and partial gastrectomy did not alter the effectiveness of orally administered cortisone. Smoother effects were usually obtained by the hormone orally.

### *Effectiveness of Corticotropin Suspensions.*

R. W. Payne, I. N. Rosenberg, M. S. Raben, A. P. Cleroux and E. B. Astwood,* Boston, Mass.

Partly purified corticotropin made in this laboratory by adsorption on cellulose was found to be therapeutically effective in a dose of 1 mg. given every eight hours. This preparation facilitated studies on different methods of administration; it could be injected intravenously and could readily be incorporated in small volumes of various media designed to prolong absorption from subcutaneous sites.

The subcutaneous injection of 1 mg. in saline was followed by a maximal urinary ketosteroid excretion in three to five hours and a return to normal or subnormal values after six to eight hours; there was a parallel clinical response. A single dose of 4 mg. suspended in 0.5 cc. of sesame oil produced an increase in ketosteroid excretion lasting 36 to 48 hours with therapeutic effectiveness over this length of time.

Used in the treatment of rheumatic and allergic diseases in a dose of 4 to 8 mg. every 24 or 48 hours, it was found to be effective in all of twelve trials when given once daily, and in six of nine trials when injected once every two days. Severely ill patients required daily injections; ambulatory patients showed satisfactory responses to 48 hourly doses for as long as treatment was given (four to six weeks).

The purified corticotropin preparation was found to be adsorbed strongly by small amounts of powdered oxidized cellulose and the aqueous suspension could be given subcutaneously. Although optimal conditions have not thus far been established, observations on seven patients indicated therapeutic effectiveness for 18 to 24 hours.

### *A Comparison of the Metabolic Effects of the Adrenal Cortical Steroids, Compounds A, E, F and S Acetate.*

Olof H. Pearson, Leonard P. Eliel and Vincent P. Hollander, New York, N. Y. (Introduced by C. P. Rhoads).

Microcrystalline, aqueous suspensions of Compounds A, E, F (Kendall) and S (Reichstein) acetate were administered intramuscularly to 4 patients with chronic lymphatic leukemia. Metabolic balances of N, P, K, Ca, Na and Cl were obtained. The steroids were given at intervals of 6 hours in a daily dosage of 100 to 800 mgs. for periods of 10 to 24 days. The effects of Compounds E and F were compared in 2 patients, Compounds E and S in one patient. Compound A only was studied in one patient.

Compound E in a daily dosage of 100 to 200 mgs. regularly produced negative balances of N, K, P and Ca, and positive balances of Na and Cl.

Compound F in a daily dosage of 100 to 200 mgs. produced negative balances of N, K, P and Ca of approximately the same order of magnitude as that obtained with Compound E. However, Compound F in amounts up to 400 mgs. per day failed to induce retention of Na and Cl.

Compound A in a daily dosage of 100 mgs. produced marked retention of Na and Cl, but in daily amounts up to 800 mgs. was without significant effect on N, K, P and Ca balances.

Compound S in a daily dosage of 200 mgs. produced slight retention of Na and Cl which was of less magnitude than that produced by similar amounts of Compound E. Compound S produced no significant effect on N, K, P and Ca balances.

Compounds E and F induced shrinkage of lymphoid tumor masses and a rise in white blood count, whereas Compounds A and S were without effect on lymphoid tissue.

These observations indicate qualitative as well as quantitative differences in the physiologic effects of the adrenal cortical steroids, as indicated by the separation of effects on protein and Na and Cl metabolism.

### *An* In Vivo *"Oxygen Dissociation Curve."* RAYMOND PENNEYS, Philadelphia, Pa. (Introduced by Hugh Montgomery).

By means of the Millikan oximeter and the oxygen (polarographic) electrode, simultaneous measurements of the arterial oxygen saturation of the blood and oxygen tension of warm skin were made *in vivo*, in normal human subjects. It has been shown previously that skin having a fast circulation has an oxygen tension approximately that of the arterial blood. The electrode used was the stationary open type platinum micro-electrode which measures relative changes in oxygen tension. The arterial saturation was decreased, or increased, by the inhalation of gases of varying oxygen concentration. A total of twenty-one experiments were done.

When the saturation was lowered to 85 per cent, the tension fell to 51 per cent of the original reading, obtained while the subject was still breathing room air. At 80 per cent saturation it was 42 per cent; at 75 per cent, 39 per cent; and at 70 per cent saturation, 35 per cent. When the arterial saturation was increased to 98 per cent, the oxygen tension rose to 135 per cent. At 99 per cent saturation it was increased to 180 per cent, and at 100 per cent saturation, to 460 per cent. When these oxygen tension and arterial saturation values are plotted against one another, a curve is evolved which is almost identical to the *in vitro* oxygen dissociation curve for blood.

The oximeter and oxygen electrode therefore allow measurements related to the dissociation of blood to be made *in vivo*. These methods require considerably less time and skill than the *in vitro* methods and give continuous, dynamic information. Applications are being made at the bedside of critically ill patients.

### *Altered Metabolic Response Prior to the Development of Hypertension in a Patient with Hypertensive Vascular Disease.* GEORGE A. PERERA,* New York, N. Y.

Evidence has been obtained previously pointing toward variations in pressor response and disturbances in salt and water metabolism in patients with essential hypertension.

A 27-year-old woman, selected because of a strong family history of hypertension and a positive cold pressor test, was found to have repeated casually-determined blood pressure values ranging between 116/72 and 134/84. Placebo injections failed to modify the arterial tension, whereas the administration of desoxycorticosterone, 10 mgs. daily for five days, was associated with a rise in pressure to hypertensive levels. In addition, the rigid restriction of salt for 24 hours while on a constant diet resulted in weight loss of but 0.28 Kilos. Followed closely thereafter, persistent hypertension developed rather abruptly three months after the above tests, with evidence by exclusion that it was on the basis of hypertensive vascular disease.

The failure to lose significant weight on salt withdrawal has been associated principally with uncomplicated hypertensive disease. An early pressor response to desoxycorticosterone has been noted before only in hypertensives. Treated for similar brief periods, no modification of arterial tension occurred in over 20 normotensive adults, including a few subjects with vasomotor hyperreactivity.

This study suggests that an altered metabolic response exists before the development of hypertension, but gives no indication as to whether such changes precede the onset of overt disease by more than a short margin. Nevertheless, it provides a further argument that hypertension is but a manifestation of disease and that further search is warranted for a metabolic factor in advance of the first outward signs of hypertensive vascular disease.

### *The Determination of Cardiac Output by a Continuous Recording System Utilizing the Injection of Iodinated ($I^{131}$) Human Albumen.* W. H. PRITCHARD, W. J. MACINTYRE, R. W. ECKSTEIN and H. L. FRIEDELL, Cleveland, Ohio (Introduced by Joseph M. Hayman, Jr.).

In an effort to find a reliable method for determination of cardiac output which could be conveniently performed at repeated intervals of several days or weeks, calculation of cardiac output involving dilution of an injected substance as a function of time has been used. Previous studies by Hamilton and Nylin have established the validity of the theory.

Utilizing iodinated ($I^{131}$) human serum albumen in small amounts (75–150$\mu$c), studies have been carried out in dogs and man to test the accuracy of application of the method based on this theory.

During the rapid injection of a few ccs. of the radioactive material, arterial blood was allowed to flow slowly through a small properly shielded tube over a scintillation counter with the gamma activity recorded continuously by a Berkeley counting rate computer used in conjunction with a scaler and Esterline Angus graphic meter.

By calculation from the dilution curve so inscribed, cardiac output was determined, and following a subsequent 10 minute interval the final dilution (blood volume) level also recorded. Specific activity of the 10 minute arterial whole blood sample was measured *in vitro* in terms of $\mu$c/cc., and the flow system calibrated by the observed response of the system *in vivo* to this known activity at final dilution.

In eight experiments on dogs the dilution method gave average values 15 per cent higher than corresponding simultaneously recorded aortic rotameter flow values. If 5 per cent is added to the rotameter values to allow for the unmeasured coronary flow, an average agreement of + 10 per cent is obtained with a range of + 5 to + 16 per cent.

Preliminary studies on nine patients using cardiac catheter technique for comparison has given satisfactory results in eight with an agreement within ± 9 per cent.

In the method discussed no phase appeared critical except that of ensuring a sufficiently rapid flow past the counter.

### *The Rate of Utilization of Radioiron as a Measure of Bone Marrow Damage from Irradiation.* J. E. Rall, Leon Hellman, Wendell Peacock and Rulon W. Rawson,* New York, N. Y.

Fourteen patients have been studied before and after isotopic radiation from $I^{131}$ calculated to have delivered from 33 rep (Roentgen equivalents physical) to 1300 rep to the blood as determined by measuring the level of circulating $I^{131}$ and calculating the rep with Marinelli's formulae. The degree of radiation damage as measured by changes in the peripheral blood was better correlated with the rep delivered to the blood than with the amount of $I^{131}$ administered. The doses of $I^{131}$ were inconsistently correlated with the rep delivered to the blood: a 300 millicurie dose of $I^{131}$, for example, delivered 188 rep and a dose of 254 millicuries delivered 765 rep to the blood. One death due to irradiation occurred after a total of 1300 rep delivered by five doses of $I^{131}$ in 14 months. Changes in the formed elements of the peripheral blood were observed similar to those described by others.

By studying radioiron utilization a decrease in the rate of hemoglobin synthesis following relatively small amounts of irradiation has been demonstrated. Four patients were given $Fe^{55}$ before and after radiation and the rate of hemoglobin synthesis measured by frequent determinations of the hemoglobin radioiron levels. The curve of increase in radioactivity of hemoglobin was a simple exponential curve from which the time for half build up ($T\frac{1}{2}$) in normal patients was 1.5 to 4 days. After irradiation $T\frac{1}{2}$ increased to 3 to 6 days. A marked decrease in the rate of hemoglobin synthesis after as little as 100 rep was noted. This occurred in spite of the fact that there were no significant changes in the hemoglobin, red blood count or lymphocyte count.

The change in the rate of hemoglobin synthesis as measured by $Fe^{55}$ was the most sensitive single index of radiation damage.

### *Antihyaluronidase Response Following Hemolytic Streptococcal Infection in Childhood.* Lowell A. Rantz* and Joseph M. Di Caprio, San Francisco, California.

The heat-stable serum streptococcal antihyaluronidase titers have been measured following Group A hemolytic streptococcal infection in a large number of infants and children using the mucin clot inhibition technique, and compared with the levels of antistreptolysin O simultaneously determined.

Three distinct patterns of antistreptolysin response in childhood have been previously reported. In one, the production of antibody was feeble and, in a second, brisk but poorly maintained. Children who exhibited either of these patterns failed to form measurable antihyaluronidase.

A third group made large amounts of antistreptolysin O and levels were well maintained for long periods of time. Moderate to very high titers of antihyaluronidase were discovered following infection in many but not all of these individuals.

Evidence can be presented which indicates that the changing patterns of antistreptolysin and of antihyaluronidase response result from the conditioning of the antibody-forming apparatus by repeated infection by hemolytic streptococci.

Antihyaluronidase may be a more feeble antigen or be produced in less effective amounts by certain strains.

This information suggests the role of repeated infection by hemolytic streptococci in the pathogenesis of rheumatic fever, since antihyaluronidase is regularly present in the serum during the acute phase of the disease.

### *Studies on Intravenous ACTH Administration.* Albert E. Renold, George Maistereena and Peter H. Forsham,* Boston, Mass.

ACTH has been administered intravenously to normal subjects and to patients with and without adrenal pathology. Quantitative relationships between dosage, duration of administration and the adrenocortical stimulation obtained have been investigated. A standard intravenous ACTH test and an assay method for ACTH in man have been evolved.

Confirming previous reports, the intravenous administration of relatively small amounts of ACTH was found to afford a rapid and maximal adrenal cortical stimulation, as measured by a fall in circulating eosinophils and a rise in urinary 17-ketosteroid excretion. No response was demonstrated in five patients following bilateral adrenalectomy. In normal subjects a straight-line relationship was established between ketosteroid excretion, ACTH dosage (over a range from 0 to 15 mg. LA-1-A) and the duration of infusion for a constant dose.

As the intravenous ACTH test, 20 mg. Armour ACTH were infused in 500 ml. diluent (saline or 5 per cent dextrose) over eight hours. The ten hour eosinophil fall was always 90 per cent or more in patients without adrenal insufficiency and the rise in twenty-four hour ketosteroid excretion (7–22 mg.) afforded an estimate of the acute functional reserve of the adrenal cortex. No anaphylactic phenomena occurred with the slow intravenous infusion of commercial ACTH.

The minimal amounts of ACTH and the quantitative relationships mentioned suggested use of the intravenous method as an assay of ACTH in man. Highly purified polypeptide mixtures and various commercial preparations have thus been compared.

Four patients "resistant" to ACTH after repeated courses of intramuscular administration showed no response either to intramuscular ACTH in doses of 25 to 50 mg. every six hours for one to four days or to a continuous intramuscular drip. They did, however, respond normally to the intravenous test. Increased tissue inactivation apparently plays an important part in acquired adrenocortical unresponsiveness.

### *The Effect of an Inhibitor Fraction from Human Sputum on the Multiplication of Influenza Virus.* HARRY M. ROSE,* New York, N. Y.

Previous studies have indicated that human sputum contains different factors which a) inhibit hemagglutination by influenza virus (PR8 strain), b) reduce infectivity of the virus for mice and chick embryos, and c) enhance infectivity of the virus for mice under certain circumstances. A fraction has been obtained from sputum which inhibits infection of mice and chick embryos but possesses no enhancing properties; the inhibitor substance in this fraction deteriorates spontaneously unless frozen or lyophilized, is heat labile and is not specific antibody. Chick embryos were inoculated with 1000 EID50 of PR8 virus by the chorioallantoic route and received one injection of the inhibitor fraction by the same route from 1 to 6 hours later; in some experiments a second injection of inhibitor was made 24 hours after the first. The amounts of infective virus and viral hemagglutinin in the chorioallantoic fluids and membranes of treated and control chick embryos were determined at daily intervals for 4 days after primary inoculation. In treated embryos the multiplication of virus in the chorioallantoic membrane was delayed and decreased and the release of virus into the chorioallantoic fluid was reduced or entirely prevented. These effects were obtained in embryos given one injection of inhibitor as late as 6 hours after infection and were magnified by two injections of inhibitor. It is concluded that human sputum contains a substance which may restrain the multiplication of influenza virus within infected host cells, and that this substance may also modify the release of virus from these cells. The action of the inhibitor substance may be compared with the effect of Friedlander bacillus polysaccharide on mumps and PVM viruses, as reported by Horsfall and Ginsberg. The chemical nature of the inhibitor is unknown but other studies suggest that it may consist at least in part of carbohydrate.

### *The Influence of Cortisone on Water Diuresis in Man.* JACK D. ROSENBAUM, ROBERT K. DAVIS and BRUCE C. FERGUSON, Framingham, Mass. (Introduced by Maurice B. Strauss).

The renal excretion of water and solutes has been studied in 9 adult male patients free of renal or cardiovascular disease before, during, and after the parenteral administration of 200 or 500 mg. of cortisone daily. With the subject sitting, water was ingested in the morning to establish and maintain a load of 1500 ml. for 3 to 8 hours. During cortisone administration, rates of urine flow reached 34 ml. per minute, exceeding control values by 50 to 200 per cent. The excretion rates of sodium, potassium, chloride, urea and creatinine after water loading were similar in treatment and control studies. The augmentation of diuresis during therapy could be diminished by decreasing the dietary intake of sodium chloride. The rate of diuresis during successive collection periods declined if the excretion rate of sodium (and chloride) also fell; after such a decline, if assumption of the recumbent posture resulted in an increased rate of sodium excretion, the rate of diuresis rose again.

During therapy, endogenous creatinine clearance often increased by as much as 100 per cent, but the magnitude of this increment bore a variable relationship to the increase in urine flow observed. Preliminary studies suggest that after therapy is instituted, $C_{Cr}$ may increase several days before augmentation of diuresis occurs and that after therapy is discontinued $C_{Cr}$ may remain elevated after the rate of diuresis has returned to control values.

Sensitivity to exogenous pitressin (15 milli-units intravenously) was unaltered.

Striking changes in the diurnal rhythm of the renal excretion of water, sodium, potassium and chloride were regularly observed. The normal nocturnal suppression of water and electrolyte excretion was often completely eliminated and always diminished. Ambulatory subjects showed an actual reversal of the rhythm; the nocturnal rates of water and electrolyte excretion exceeded those of the day.

### *The Mechanism of Anemia in Leukemia and Malignant Lymphoma.* J. F. ROSS,* C. L. CROCKETT, JR. and C. P. EMERSON, Boston, Mass.

The anemia developing in patients with leukemia, lymphoma and other neoplastic diseases is obscure as to mechanism and pathogenesis. The usually accepted concept that it is due to the mechanical crowding out of erythropoietic tissue by neoplastic cells is not, in the majority of cases, confirmed by anatomic study. Neither does blood loss, an overt hemolytic process, or "toxic" bone marrow depression adequately explain the observed anemia. We have investigated the possibility that it may be caused by an increased rate of destruction of red cells.

We have evaluated the rate of blood destruction by simultaneously: 1) determining the quantitative fecal urobilinogen and calculating the hemolytic index from $P^{32}$-determined red cell mass; 2) and by measuring the rate of disappearance of transfused normal erythrocytes in leukemic patients by methods of selective red cell agglutination.

In ten patients with leukemia or lymphoma it has been demonstrated that the rate of destruction of erythrocytes is markedly increased. The differential agglutination studies indicated a rate of disappearance of transfused cells two or three times more rapid than normal, and the hemolytic index was elevated considerably in the majority of subjects. Evidence was also obtained that the rate of formation of red blood cells was often increased. Only one of this group had the usual evidences of hemolytic disease such as reticulocytosis, hyperbilirubinemia or a positive Coombs test.

These observations indicate that the anemia of leukemia and malignant lymphoma is due in large part to an increased rate of red blood cell destruction, and in many instances, in contrast to the generally accepted concept, the rate of red cell formation, instead of being diminished, may actually be accelerated.

### *Relation of Abnormal Serum Components to Bence Jones Protein in Multiple Myeloma.* R. W. Rundles,* G. R. Cooper and R. W. Willett, Durham, N. C.

The significance of the abnormal protein components in the sera of patients with multiple myeloma has been studied in a series of 30 patients with the disease. Methods used included electrophoresis, diffusion and ultracentrifugation.

Abnormal serum components, recognizable in the patterns of 25 of the 30 patients, varied from minute increments to as much as 7–9 gm. per 100 cc. The electrophoretic mobility of the serum components, 0.3–2.6, was equal to or less than that of the Bence Jones protein excreted by the same patient. Differences in mobility were confirmed by adding urinary protein to serum and by studying mixtures of Bence Jones proteins.

There was no relation from patient to patient between the amount of abnormal protein in the serum and the amount of Bence Jones protein excreted in the urine, but in individual patients the serum increment remained proportional to the amount of Bence Jones protein in the urine as plasma cell growth was suppressed by therapy or as relapse occurred.

Abnormal serum components had diffusion rates $D^{\alpha}_{20,W} = 3.1–5.0$ (in 10 patients) and sedimentation velocities $S^{\alpha}_{20,W} = 6.19–6.76$ (in 14 patients). Calculated molecular weights ranged from 120,000 to 200,000, the majority between 140,000 and 160,000.

Bence Jones urinary proteins were variable in diffusion rate, $D^{\alpha}_{20,W} = 4.7–9.8$, and sedimentation rate, $S^{\alpha}_{20,W} = 2.44–4.40$. The calculated molecular weight of Bence Jones proteins excreted by 10 patients was 24,000 (1), 32,000–42,000 (4), 45,000–52,000 (3), 60,000 (1), and 90,000 (1).

Our findings indicate that the serum increments in myeloma are homogenous abnormal proteins of high molecular weight. Bence Jones proteins are moieties of low molecular weight, filterable through the glomeruli, apparently derived from the abnormal serum components.

### *A Safe Immunologic Adjuvant for Enhancing the Height and Persistence of Antibody Response to Influenza Virus Vaccines in Man.* Jonas E. Salk,* Mary Lynch Bailey, and Angela M. Laurent, Pittsburgh, Pa.

The results of field trials on influenza virus vaccines indicate the need for enhancing further the effectiveness of this immunizing agent. Various methods have been employed in attempts to accomplish this, i.e., multiple inoculations, intracutaneous rather than subcutaneous injections, increasing virus concentration in the vaccine, and the addition of certain immunologic adjuvants. Of these, only the use of the mineral oil adjuvant of Freund has yielded data sufficiently worthwhile to pursue further. When the Henles tried such adjuvants in humans, using the emulsifying agent Falba and mineral oil, they encountered abscesses in two out of eighty subjects and persistent nodules in the remainder. This discouraged further use of this preparation in man. However, in the course of other studies in this laboratory, using a different mineral oil (Bayol F) and another emulsifying agent (Arlacel A), it was observed in monkeys that when such mixtures were inoculated intramuscularly, no undesirable reactions were observed clinically. This prompted the extension of these studies to the problem of influenza virus vaccine, both in monkeys and in man, with very dramatic results in terms of enhancement and persistence of antibody response without undesirable side reactions. In addition, it was found that the amount of antigen could be reduced to very low levels and when combined with adjuvant is still effective in inducing the formation of high levels of antibody. As of this writing, more than two hundred individuals have been inoculated with such preparation without untoward effect and immunologic data over a six month period, in some, indicate a high degree of persistence of antibody well beyond the range achieved with vaccines in use at present. A further effect has been the enhancement of antibody response over a broad range of the antigenic spectrum, especially for the type A strains that vary so widely in antigenic composition.

### *Some Observations on the Mechanism of Delirium in Pernicious Anemia.* Donald C. Samson, Scott N. Swisher, Richard M. Christian and George L. Engel,* Rochester, N. Y.

Psychological disturbances in pernicious anemia are well known. Examination of 12 consecutive patients admitted to our hospital revealed delirium to be the most common disturbance, occurring in 11. One of the 11 was also schizophrenic.

In delirium the basic psychological disturbance is in level of awareness presumably due to some disturbance in cerebral metabolism. The purpose of this paper is to examine the relationship between the pernicious anemia process and the delirium.

Techniques used were serial mental status examinations particularly directed toward evaluation of the level of awareness; serial EEG's with quantitative frequency analysis; and the usual hematological studies.

Results: (1) 11 of 12 patients were delirious by clinical criteria at some time; (2) 8 of these 11 had slowing of the EEG consistent with delirium, 2 had records at lower limit of normal frequency but which became faster with clinical improvement. One had low voltage fast type record; (3) all 12 patients responded hematologically, some suboptimally, to treatment. One of these patients had improvement in mental status, EEG and RBC, but with no reticulocyte response, on transfusions alone. Of the 11 clinically delirious patients, 9 recovered completely from delirium, one improved, and one was unchanged. Because their clinical condition became much worse two days after admission, 2 patients did not have serial EEG's. Of the 10 patients who improved, there was a correlation between onset of mental status improvement and the reticulocyte response in 8. In the 7 cases in which quantitative EEG studies could be done, EEG improvement correlated with the reticulocyte response and preceded the RBC rise in 5; one patient had clinical improvement without EEG change; one patient responded to transfusion alone without reticulocyte response.

From this study the authors suggest the following: (1) that disturbance in cerebral function is common in pernicious anemia; (2) that this disturbance is a primary metabolic one and not necessarily secondary to the anemia; (3) that it is reversible with specific therapy in most instances.

### *Studies of Coronary Disease in the Experimental Animal. III. Polarographic Studies of Intramyocardial Oxygen Availability in Dogs with Acute Coronary Occlusion and Narrowing, Correlated with Epicardial Electrocardiograms.* JOHN J. SAYEN, WARNER F. SHELDON, HARRY F. ZINSSER, P. T. KUO, ORVILLE HORWITZ and DAVID P. MCCALLIE, Philadelphia, Pa. (Introduced by Charles C. Wolferth).

Local oxygen availability in exposed dog hearts has been studied by an improved technique permitting readings from ten platinum electrodes per minute or continuous readings at individual sites. Based on electrode values during coronary occlusion, three myocardial zones could be distinguished: 1) "Central" areas, showing rapid falls in electrode readings to less than a quarter of control values, 2) "Border" areas, falling more slowly and to a lesser degree, 3) "Remote" areas which show no significant change during occlusion. This physiological classification of myocardial areas by electrode values was consistent with the gross appearance of the heart when the ischemic regions' borders could be discerned. The earliest epicardial electrocardiographic changes, even at the center of ischemic areas, were much less definite than the conspicuous changes in oxygen electrode values, which appeared within ten seconds.

Responses to pure oxygen inhalation before release of coronary occlusion were as follows: 1) electrode rises in central areas were rare, slight and transient, 2) border area electrode values regained more than a third of their initial fall in 68 per cent of instances, 3) remote electrodes regularly recorded large increases characteristic of normal muscle.

Acute coronary narrowing had effects similar to occlusion but permitted more time to study the ischemic areas. Larger areas of the ischemic muscle behaved like "border" zones and consequently electrode rises were more frequent during oxygen administration before release of the narrowing. Electrocardiographic abnormalities in the border area sometimes tended to diminish when the oxygen electrode showed a rise in the same area. Fifty per cent oxygen inhalation produced much smaller increases than did pure oxygen.

Conclusion: Platinum electrodes provide a fairly precise, very sensitive means for studying localized myocardial ischemia and responses to oxygen inhalation. These experimental data support the inference that human myocardial infarction should be treated by measures producing maximal alveolar oxygen concentrations.

### *Leptospiral Meningitis. Investigation of a Water-Borne Epidemic Due to L. pomona.* MORRIS SCHAEFFER, Montgomery, Ala. (Introduced by Paul B. Beeson).

In July of 1950, in the vicinity of Geneva, Alabama, a rural, peanut growing area, there occurred an epidemic of an influenza-like disease, attacking about 50 of approximately 80 adolescents and young adults who had been swimming in the creek. An artificially enlarged portion of it was frequented as the local swimming hole. The summer had been hot and dry and the water shallower and slower moving than usual. Dead hogs had been seen floating in the creek and its branches. On July 4 the hole was crowded with swimmers. Two to 10 days later they began to develop chills and fever, headache, nausea, vomiting, arthralgia, myalgia, stiff neck and back. Many had conjunctivitis and photophobia, some chest pain, abdominal pain, constipation or diarrhea, a few transient maculo-papular rashes. None showed jaundice or other liver involvement. Spinal fluid findings on several were typical of aseptic meningitis. The duration of illness was 5–14 days with occasional relapses but no complications or sequelae.

Because of the abundance of rats and hogs on the outlying farms, agglutinations for leptospira were run on the paired serum specimens obtained from 22 of the involved individuals after possible viral and rickettsial agents were ruled out. Of these 18 showed antibody titer increases against *L. pomona* in a magnitude attributable to recent infection with this agent. A control group of sera from individuals of the same age group and locality but not swimming nor ill was negative.

Since leptospirosis was not suspected at the outset, no attempts at isolation of leptospira were made and the water samples, frozen for virus isolation, were unsuitable. Animal surveys could not be made until 3 months later when there had been considerable turnover especially among the hogs. However, some positive sera were found among the few remaining hogs as well as cows, horses and mules.

An extensive rat survey yielded negative cultures and serum agglutinations for *L. pomona*.

This is the first description of such an epidemic in the United States. A similar outbreak due to *L. grippotyphosa* was reported recently to have occurred in a group of military personnel in France.

### *The Effects of Intravenous Papaverine and Procaine on Cerebral Circulation and Metabolism.*

P. Scheinberg, H. W. Jayne, M. Rich and M. S. Belle, Coral Gables, Fla. (Introduced by Frank L. Engel).

Adequate therapy of many types of cerebral vascular accidents will depend upon agents which produce dilatation of cerebral vessels. In this study the nitrous oxide technique for determining cerebral blood flow was used to measure the effects on the cerebral circulation of large intravenous doses of papaverine in one group of patients and procaine in another group. All age groups are represented in the study; patients with known cerebral vascular disease were not included. Control studies were done prior to administration of the drug.

Intravenous papaverine (180 mgm.) resulted in a 24 per cent increase in cerebral blood flow, a 21 per cent decrease in cerebral arterio-venous oxygen difference, a 28 per cent decrease in cerebral vascular resistance, and a 12 per cent decrease in mean arterial pressure. Cerebral oxygen consumption was unchanged. Cerebral venous oxygen tension was slightly increased. The decrease in cerebral vascular resistance and increased blood flow are indicative of dilatation of cerebral blood vessels, though the mechanism for this is not yet known. It should be noted that facial flushing was insignificant in these subjects. It was found that administration of papaverine must be accomplished over at least a 15 minute interval. Too rapid infusion of the drug may cause extreme drops in blood pressure and great apprehension on the part of the patient, with changes in cerebral circulation that cannot be attributed directly to the drug.

Procaine (750–1000 mgm.) administered intravenously over a period of 12 to 15 minutes produces no significant alteration in any of the cerebral metabolic functions, though our data indicate that further studies may show an increased cerebral vascular resistance following procaine.

### *The Effects of Adrenocorticotropic Hormone (ACTH) on Cerebral Blood Flow and Metabolism.*

James F. Schieve, Peritz Scheinberg and William P. Wilson, Durham, N. C. (Introduced by W. M. Nicholson).

While patients receiving adrenocorticotropic hormone (ACTH) usually show an increased sense of well-being and mild euphoria, a small number become psychotic. These clinical observations led to a study of cerebral blood flow by the nitrous oxide method. The oxygen consumption and glucose utilization per 100 gm. brain were obtained by multiplying the blood flow by the respective arterial-cerebral venous differences.

A total of 44 observations on 14 patients was made (15 before, 23 during, and 6 following ACTH). ACTH was given in full therapeutic doses (60–100 mgs. Armour or 30–40 mgs. Wilson daily). One 12 year old child received 25 mgs. Armour daily. Measurements were made on therapy at least once and as often as four times at intervals varying from 7 to 56 days.

On ACTH a significant reduction in cerebral blood flow occurred (61 to 50 ml./minute/100 grams brain). This was accompanied by a significant widening of arterial-cerebral venous oxygen (5.8 to 6.5 vols. %) and glucose (8.2 to 10.1 mgs. %) differences. These were of sufficient magnitude to result in essentially unchanged cerebral oxygen consumption (3.5 to 3.2 ml. $O_2$ per 100 gms. of brain per min.) and glucose utilization (4.9 to 4.9 mg. glucose per minute per 100 gms. of brain). A 32 per cent increase in cerebral vascular resistance occurred, but only a 9 per cent increase in mean arterial blood pressure.

Six patients with initially low flows (below 50) with low $CMRO_2$ (2.8) and CMR Gl. (3.6) showed no change in cerebral blood flow or metabolism on ACTH in spite of the usual clinical response.

Two patients who became psychotic on ACTH revealed no change in cerebral metabolism or blood flow as measured by this method.

### *Comparative Studies of the Biochemistry of Heart and Skeletal Muscle.*

Gerhard Schmidt, Maria Fuld, Ricardo Cubiles and Samuel Proger,* Boston, Mass.

Both the skeletal and heart muscle of higher animals resemble each other with regard to their content of some characteristic constituents, for example, myosin and adenyl-pyrophosphate. A study of the literature, however, reveals that they differ strikingly in many important aspects of metabolism and of chemical composition. As examples there are the well-known differences in content of substances such as cytochrome C, aldolase, and phosphocreatine, as well as the striking difference in the behavior of these tissues under anaerobic conditions.

Because of the possible relationship of demonstrable biochemical differences to the special functional characteristics of heart and skeletal muscle a comparative study of both tissues has been undertaken. Thus far our studies have dealt with the content of phosphorylase and phosphoglucomutase of both tissues (in rats, rabbits, and dogs) as well as the content of bound and free $\beta$-alanine in the heart and skeletal muscle of rats and beef.

The phosphorylase content of heart muscle was found to be roughly about half that of skeletal muscle while the phosphoglucomutase content showed a much greater difference in the two tissues.

Bound $\beta$-alanine in the form of carnosine and anserine constitutes, next to phosphocreatine, the predominant component of the nonprotein nitrogen fraction of skeletal muscle. On the contrary the heart muscle was found to contain only negligible quantities, if any, of free or bound $\beta$-alanine.

Since the total nonprotein nitrogen fraction is quantitatively similar in both tissues and since the heart muscle contains only about ⅒ the amount of phosphocreatine as is found in skeletal muscle as well as practically no

$\beta$-alanine compounds, it must be concluded that there exists a large content of nonprotein nitrogen substances peculiar to the heart muscle. We are at present attempting to determine the nature of these substances.

### *Relative Viscosity and Clotting Time of Whole Blood in the Healthy and in Those with Vascular Disorders.* ROBERT A. SCHNIEDER, New York, N. Y. (Introduced by Harold G. Wolff).

Although the importance of an increase in blood viscosity coexisting with a shortened clotting time would be readily recognized, no definite studies of such simultaneous measurements in human subjects are available. Accordingly such measurements were undertaken on individuals subjected to a variety of stresses including 20 healthy subjects, 20 patients with essential hypertension, 8 patients with structural heart disease undergoing congestive failure and 5 patients with thrombophlebitis. Clotting time was measured in siliconized tubes and relative viscosity was determined using a capillary viscosimeter.

In healthy subjects shortened clotting time was uniformly accompanied by a variable increase in blood viscosity. In certain cases hematocrits were determined and the increase in viscosity appeared related to an increase in red cell mass. Twenty hypertensive patients were studied because of their known proclivity for thrombosis. These subjects reacted uniformly during stressful interviews productive of a pressor response with a shortening of the clotting time (average 15%) and an increase in viscosity (average 7%). Five hypertensive subjects, in whom no pressor response occurred, showed no changes in viscosity or clotting time. Patients with heart disease showed shortening of clotting time and increase in blood viscosity during periods of congestive failure which values approached normal with compensation. In contrast, patients with thrombophlebitis showed shortened clotting time but usually no increase in viscosity during bouts of venous thrombosis. These patients were anemic and this may have accounted for their failure to show a rise in blood viscosity.

The factor of viscosity is ignored in calculating peripheral resistance, the assumption being that it is constant. This study indicates that this convention should be reexamined and suggests that variations in blood viscosity may be pertinent to the hemodynamics of hypertension. Shortened clotting time is often accompanied by increased viscosity and this may bear upon the problem of thrombosis.

### *Studies on the Sulfhydryl Content of the Serum Proteins.* EMANUEL B. SCHOENBACH * and NORMAN WEISSMAN, Baltimore, Md.

The biological significance of sulfhydryl containing compounds has been explored since glutathione was first isolated from yeast in 1921. Evidence has been presented that the sulfhydryl groups are important for the structure of proteins and various metabolic processes such as cellular division, and enzyme activity.

An amperometric method has been adapted to the determination of sulfhydryl groups in the serum proteins. This technique has been shown to be quantitative and specific for the $-$SH group. This determination is not affected by the presence of creatine, desoxyribonucleic acid, penicillin or cystine. Previous studies on sera from normal individuals and patients suffering from various types of illness have shown that the protein sulfhydryl was significantly reduced among the latter group. A further investigation was therefore initiated in which the sera were fractionated by the Pillemer procedure into albumin and globulin components and these components were analyzed with respect to their nitrogen, peptide and sulfhydryl content.

The sera from patients with carcinoma, multiple myeloma, lupus erythematosus, nephritis, cirrhosis, and one cystinuric, when thus studied, showed that the albumin was usually reduced while the globulin was normal or increased as measured by their nitrogen or peptide content. The ratio of peptide per mg. of protein nitrogen for these components did not deviate markedly from the normal. The sulfhydryl per gram of albumin nitrogen or per peptide bond was below the normal value among 86–87% of the samples and the sulfhydryl per gram of globulin nitrogen or peptide bond was low among 76–79%. It is suggested that a common metabolic abnormality related to protein formation which results in a qualitative change in both albumin and globulin components occurs in these disease states which is reflected in the low sulfhydryl values.

### *Effects on Hypertension of Sulfhydryl and Hydrazine Compounds.* HENRY A. SCHROEDER, St. Louis, Mo.

Pherentasin, a sustained pressor substance found in human hypertension, is a primary amine containing a carbonyl group, the latter apparently being necessary for prolonged activity. This formula suggests that two metabolic disturbances may be associated with hypertension: imperfect deamination of amines and incomplete reduction of certain ketones. To test the latter hypothesis, a group of sulfhydryl-containing compounds were injected in brief experiments in normotensive and hypertensive rats. Differential prolonged depressor action was exhibited in the hypertensive animals by substances such as cysteine, dimercaptopropanol, $\beta$-mercaptopropionic acid, mercaptosuccinic acid, thioglycollic acid and others of similar configuration. When the sulfhydryl group was on a ring structure no such action was demonstrated; sulfur containing compounds such as cystine, methionine, oxidized glutathione and the like exhibited no action. The effects lasted 2 to 3 hours in rats and dogs; in man dimercaptopropanol likewise exerted a brief (2 hr.) hypotensive action. These substances abolished the pressor action of arterenone but not of arterenol, and probably destroyed pherentasin in like manner.

Because hydrazine compounds also may attack carbonyl groups, two substances, 1-hydrazino-phthalazine and 1-hydrazino-4-methyl-phthalazine were tested for their hypotensive properties in renal hypertensive dogs; prolonged chronic lowering of blood pressure was observed. In 30 patients with hypertension these agents given by mouth exerted continuous hypotensive effects for days or weeks; the most pronounced actions occurred in neurogenic hypertension, the least in renal hypertension. Adverse renal effects were not observed. Previous sympathectomy enhanced the action considerably. Tachyphylaxis occasionally occurred, but was not persistent. Toxic manifestations of headache and nausea could be controlled by anti-histaminic drugs and soon disappeared. Postural hypotension accompanied initial administration. It is believed that these two types of compounds are probably anti-hypertensive agents.

### *Crystalline Calcium Chondroitin Sulfate.* MAXWELL SCHUBERT and JULIA EINBINDER, New York, N. Y. (Introduced by Colin M. MacLeod).

Mucopolysaccharides are major components of connective tissue and information that will help to characterize and to distinguish them is needed. Of particular interest for the chemistry of joints is the chondroitin sulfate of cartilage.

Using mild isolation methods previously worked out and described, it has now been found possible to separate chondroitin sulfate from the cartilage of nasal septum as a calcium salt into fractions differing slightly in solubility and optical rotation. The least soluble fraction is amorphous, the more soluble fractions are crystalline. All have the same composition as regards hexosamine, hexuronic acid, calcium and sulfur content. This is the first time any connective tissue mucopolysaccharide has been crystallized and it is the first indication that chondroitin sulfate from cartilage may not be a single chemical substance.

Analytical data on these fractions show that the values for hexosamine, hexuronic acid, calcium and sulfur are all lower than would be expected on the basis of the known components of chondroitin sulfate. This evidence may indicate the presence of an as yet unidentified component of chondroitin sulfate.

A study has been made of the consumption of periodate by the two crystalline fractions of chondroitin sulfate. There is a slow and progressive reduction of periodate over a period of 80 hours which shows no break that could be related to the end groups, though this method has been used by Meyer, Odier and Siegrist.

### *The Effect of Pituitary and Adrenal Hormones on the Metabolism and Excretion of Potassium.* DONALD W. SELDIN, LOUIS G. WELT and JOSEPH CORT, New Haven, Conn. (Introduced by Charles H. Burnett).

In experimental animals and man, administration of ACTH or adrenal steroids may be followed by three changes in potassium metabolism: potassium excretion increases; the serum concentration falls; and hypochloremic alkalosis develops. The following study attempts to ascertain whether these changes are caused by direct action of adrenal hormones, either on tissue cells or renal tubules, or whether they are the passive consequence of sodium retention which these hormones sometimes produce.

Patients with and without edema, maintained on diets nearly constant in potassium, but containing only negligible quantities of sodium, were given prolonged courses of DOCA (20 mg per day for one month), cortisone (100–300 mg per day for 33 days), and ACTH (160 mg per day for 19 days). No change in serum concentration of potassium, sodium, bicarbonate, or chloride was detected. Potassium excretion was not accelerated by DOCA, cortisone, or ACTH, except for a transient increase during the first day of administration of the latter two hormones.

The study was extended to rats maintained on sodium-free diets containing adequate quantities of potassium. Prolonged injections of DOCA produced no change in potassium excretion, muscle composition, or serum concentrations of bicarbonate, chloride, potassium, or sodium.

The following hypothesis is therefore proposed. DOCA has no *direct* effect on internal exchanges or renal excretion of potassium; if subjects have access to sodium salts, however, a potassium diuresis may follow DOCA administration as a passive consequence of sodium retention; when potassium excretion is thus accelerated, potassium is lost as potassium chloride, thereby producing a hypochloremic alkalosis. Cortisone and ACTH appear to behave like DOCA in promoting potassium excretion by sodium retention, although these hormones in addition accelerate potassium losses by direct effect on tissue cells as well. In the doses used in these studies this cellular effect was only transitory.

### *The Role of Somatotrophic Hormone (STH) in the Production of Malignant Nephrosclerosis, Periarteritis Nodosa and Hypertensive Disease.* HANS SELYE,* Montreal, Canada.

Experiments on female piebald rats revealed that electrophoretically pure somatotrophic hormone (STH) produces nephrosclerosis with variable degrees of nephritis, marked polyuria, myocarditis, pancreatic periarteritis nodosa and hypertension. The experimental animals were sensitized to mineralo-corticoid actions by unilateral nephrectomy and an excess NaCl intake, but this sensitization in itself did not produce such changes.

The type of the toxic manifestations, as well as the sensitization of the animals to these STH effects by unilateral nephrectomy and NaCl, parallel our previous findings in animals receiving excesses of desoxycorticosterone acetate (DCA), or lyophilized anterior pituitary (LAP) material.

It is assumed that STH is the active principle responsible for the toxic actions of LAP upon the kidney and the cardiovascular system. The lesions caused by STH are considered to be, in all likelihood, secondary to a sensitization of the tissues to DCA-like mineralo-corticoids. In addition STH may also increase the production of mineralo-corticoids by the adrenals.

The cardiovascular and renal damage normally caused by STH overdosage is prevented if cortisone is simultaneously administered in doses adequate to produce adrenocortical atrophy.

of the time required to produce a definite hyperemic reaction on application of standard pressure (threshold time), which was always greater than 120 seconds and usually indefinitely prolonged, as well as equal delay in the time of disappearance of hyperemia (clearing time). In early shock and in severe dehydration less prolonged threshold and clearing times were obtained.

These results suggest that hemorrhagic shock 1) may cause only slight reductions in blood flow through the normal liver, 2) initiates prompt adrenal-cortical reaction as evidenced by fall in eosinophiles.

### *Transfer Rates Across Muscle Cell Membranes in Man: Measurement of Blood Flow and Fluid Compartments in Skeletal Muscle.* Kenneth L. Zierler, Reubin Andres and Joseph L. Lilienthal, Jr.,* Baltimore, Md.

The Stewart principle has been adapted to measurement of peripheral blood flow in the forearm of man. A suitable substance is infused into a brachial artery at *constant* rate, I mg. per min., by a motor-driven syringe delivering about 0.1 ml. per min. Blood is collected continuously from a catheter placed in a deep ipsilateral vein in the forearm. If P mg. per ml. is the concentration of infusate in venous plasma at equilibrium (corrected for recirculation), then plasma flow equals I/P. Theory does not require that infusate be confined to intravascular space, and simultaneous infusion of T-1824 and inulin have yielded identical rates of plasma flow. The method is sensitive to rapid changes in blood flow and permits continuous flow measurements. Measurements in normal man yielded flow rates of an order similar to but larger than values reported from plethysmography.

With this method it has been possible to measure rates of transfer of substances into or out of forearm muscles. Net transfer of a metabolite is a function of the product of its arterio-venous concentration difference and the rate of arterial flow to the part. Although these two variables define alterations in transfer rates induced during the course of an experiment, a reference base is essential for comparison of one individual with another.

Theoretical considerations indicate the feasibility of using the volume of muscle water in the forearm as a reference base. Muscle water might be measured as the difference between inulin and antipyrine spaces, determinable from the exponential rate of disappearance of infusates from venous effluent after halting the injection. Similar treatment of rate of disappearance of T-1824 permits estimation of plasma volume in the part; in normal individuals it constitutes some 3.5 per cent of the forearm with the wrist and hand occluded.

### *Body Water, Body Chloride and Plasma Volume Changes in Patients Receiving ACTH Therapy.* Morris Ziff and Jerome Simson, New York, N. Y. (Introduced by Currier McEwen).

In the course of adrenal cortical hormone therapy, a syndrome of hypochloremia with alkalosis has been frequently observed. It was of interest, therefore, to study changes in body chloride content during ACTH therapy. Simultaneous measurements of total exchangeable body chloride, total body water and plasma volume were made on six patients with rheumatic diseases receiving ACTH. Body water was measured by the antipyrine method and exchangeable body chloride by the distribution of bromide. Studies were carried out on each individual before, during and after treatment.

All patients retained water promptly. Two subjects, who developed edema, retained water and chloride in a parallel manner, assuming that chloride was retained in the concentration present in the extracellular fluid. Two patients, in whom the balance of chloride to water gained, calculated on this assumption, was initially positive, developed negative balances after several weeks of therapy. One patient, studied three weeks after starting therapy, demonstrated a marked negative chloride to water balance. One patient showed a persistently negative chloride to water balance throughout therapy.

Evidence of circulatory decompensation occurred in one individual, with marked increase in plasma volume. Plasma volume increased moderately in two others without overt signs or symptoms. In three, the plasma volume fell during therapy although there was a tendency for serum $K^+$ and $Cl^-$ to fall, and $HCO_3^-$ to rise, no definite metabolic alkalosis was observed.

Changes in plasma volume appeared to be dependent on repair of anemia during ACTH therapy and expansion of extracellular fluid volume. Factors influencing the relationship between changes in body water and body chloride during ACTH administration are discussed.

# INVESTIGATION OF GLUCURONIC ACID METABOLISM IN HUMAN SUBJECTS[1, 2]

By W. H. FISHMAN, M. SMITH, D. B. THOMPSON, C. D. BONNER, S. C. KASDON, and F. HOMBURGER

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(Submitted for publication December 13, 1950; accepted April 9, 1951)

Recent interest in the metabolism of glucuronic acid has been aroused mainly as a result of the realization (1) that the conjugation of glucuronic acid is an important step in the metabolism of estrogenic and steroid hormones (2, 3) and of epinephrine (4) and following the reports that glucuronic acid may be useful in arthritis (5). Although the mechanism of formation of glucuronides has been observed chiefly in studies of drug metabolism and has been widely accepted as a process of detoxication, the view is now prevalent (6, 7, 8) that this reaction represents a physiological process which we consider a "metabolic conjugation" (1). Thus, it is believed that exogenous drugs are conjugated with glucuronic acid only by virtue of certain of their chemical groupings (phenolic or alcoholic hydroxyl radicals), characteristic of the endogenous metabolites which are physiologically conjugated with glucuronic acid.

In the past, studies on glucuronic acid metabolism have been carried out almost exclusively in animals and usually in these investigations the conjugated glucuronide was isolated or determinations of urinary glucuronic acid were made (9). It is obvious that determinations of glucuronic acid in blood values would be of great value not only in animal studies but also in investigations performed in man. Accordingly, a combination of blood and urine data on glucuronic acid was considered to be necessary in any study designed to evaluate the physiological significance of glucuronic acid.

The present paper is divided into three sections. The first deals with the description of the method for determining glucuronic acid and its clinical application. The second concerns the development of a glucuronic acid tolerance test in humans, and the third describes some clinical observations relating glucuronic acid and β-glucuronidase to arthritis and cancer of the breast.

## SECTION I. DETERMINATION OF GLUCURONIC ACID IN BLOOD AND URINE

The available methods for determining blood glucuronic acid were found to be unsuited for the purpose which we had in mind, *i.e.*, the performance of as many as 100 determinations a day with reasonable accuracy. In 1943, Ratish and Bullowa (10) adapted the method of Maughan, Evelyn and Browne (11) for urinary glucuronic acid to the determination of glucuronic acid[3] in blood. An aliquot of a tungstic acid filtrate of blood was heated with a mixture of naphthoresorcinol and hydrochloric acid for 45 minutes. After cooling, the violet pigment was extracted, using a mixture of alcohol and ether, and the optical density of the ether layer read at 565 mμ in a photoelectric colorimeter. The per cent error ranged from − 11 to + 21.2. The procedure of Deichmann (12) in 1946 was performed on plasma (without deproteinization) with the naphthoresorcinol reagent, utilizing a heating period of two hours and extraction of the pigment with ether alone. Although as a rule satisfactory recoveries of glucuronic acid were obtained when it was added to plasma, frequently these were as low as 20 to 30 per cent. The use of

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[1] This work was supported by grants-in-aid from the National Cancer Institute, Bethesda, Maryland, the Damon Runyon Memorial Fund for Cancer Research, the Commercial Solvents Corporation, New York, and by an institutional grant of the American Cancer Society, Inc., New York.

[2] Presented in part at the 119th annual meeting of the American Chemical Society, April 1, 1951, Boston, Mass.

[3] The possibility that the naphthoresorcinol reaction may measure galacturonic acid as well as glucuronic acid has been pointed out by Deichmann and Dierker (12). However, in view of the fact that galacturonic acid has not yet been identified as a normal constituent of human blood and urine, it is permissible to consider the naphthoresorcinol reaction applied to protein-free filtrates of blood as one measuring essentially glucuronic acid.

ether in these procedures has the following disadvantages: rapid evaporation, especially in the summer, a strict requirement of purification and dehydration (10, 11), instability upon exposure to sunlight (12), and the presence of a serious fire hazard.

It was found possible to combine the best features of the available methods and to substitute toluene for ether as the solvent for extracting the colored product of the naphthoresorcinol-glucuronic acid reaction. In this manner, a procedure has been developed which is reasonably accurate and reliable, and possesses the advantage of convenience and safety.

## REAGENTS REQUIRED

10 per cent sodium tungstate solution

0.66 N sulfuric acid solution

0.2 per cent naphthoresorcinol solution. (A fresh solution was made for each day's determinations, employing the product obtained from Schwartz Laboratories, Inc., New York. Shaking the naphthoresorcinol water mixture in a glass-stoppered cylinder for 10 minutes effected solution.)

Toluene, Merck C. P.

Hydrochloric acid, concentrated, Merck C. P.

## METHOD OF ANALYSIS

(*a*) *Blood.* Two cc. of well-mixed oxalated blood were pipetted slowly into 14 cc. of distilled water in an Erlenmeyer flask, the contents being shaken during the process. Then, 2 cc. each of 10 per cent sodium tungstate and 0.66 N $H_2SO_4$ were added and the whole mixture agitated to ensure complete mixing. After 10 minutes it was filtered. Three cc. quantities of this filtrate representing a 1 to 10 dilution of the blood, were transferred to two duplicate boiling tubes,[4] of the dimensions of the ordinary N. P. N. boiling tubes, but fitted with ground glass stoppers. To these were added 2 cc. of 0.2 per cent naphthoresorcinol solution and 2 cc. of concentrated hydrochloric acid solution. The contents of the tube were mixed well and glass stoppers inserted. These tubes, arranged in a suitable metal rack, were placed in a gently boiling steam-heated water bath (temperature 98° C) for 60 minutes. They were then immersed in cold water (temperature + 5° C) for 10–15 minutes. After cooling, 5 cc. of 95 per cent alcohol were added to each tube, after which they were shaken to dissolve the pigment produced during the heating reaction. Then, 8 cc. of toluene were added and the tubes restoppered and vigorously shaken for one minute, this time to extract the violet pigment into the toluene phase. The aqueous layer was removed completely by suction, using a tube drawn out to a capillary attached to an aspirator and a collecting bottle. The toluene extracts were then transferred carefully to clean Evelyn cuvettes. After allowing them to stand 45 minutes in the dark to permit the extracts to clear, the transmission of light was measured in the Evelyn photoelectric colorimeter at 565 mμ using the 6 cc. well.

(*b*) *Urine.* Usually a 1 to 50 dilution of the urine was prepared with water. In urine rich in glucuronic acid a 1 to 100 dilution was employed. The deproteinization procedure was omitted, and the naphthoresorcinol and HCl solutions were added directly to 3 cc. of diluted urine. Readings in the colorimeter were made in the same way as for blood.

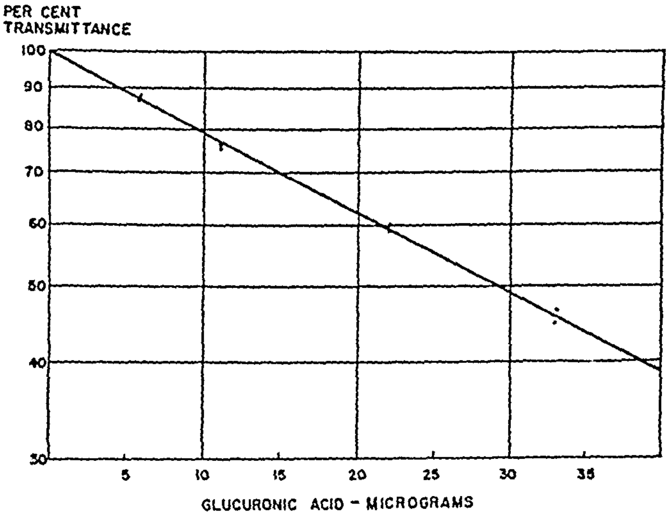

FIG. 1. RELATIONSHIP BETWEEN THE LIGHT TRANSMITTANCE OF THE GLUCURONIC ACID-NAPHTHORESORCINOL REACTION PRODUCT AND GLUCURONIC ACID CONCENTRATION

*Blanks and controls.* Blanks contained 3 cc. of $H_2O$ in place of blood filtrate or urine. It is desirable to include a standard (20 γ glucuronic acid) with each day's determinations. The optical density of this standard should read within 5 per cent of the expected optical density.

*Preparation of standard curve.* This (Figure 1) was constructed by correlating per cent transmittance with the content of glucuronolactone expressed as glucuronic acid in the standard solutions. It was found that the standard curve was completely reproducible. A curve should be constructed whenever new reagents (*e.g.*, concentrated HCl or naphthoresorcinol) are employed.

*Calculation of results.* The amount of glucuronic acid obtained by substituting the Evelyn reading of the unknown into the standard curve is termed γ G. A. The following expressions apply then to blood and urine:

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[4] These may be purchased from Macalaster Bicknell Co., Cambridge, Mass. The following washing procedure was found necessary: the tubes were rinsed thoroughly after the analysis with alcohol and tap water before being placed in cleaning solution. The use of a wax pencil for marking these tubes is prohibited, since any wax which finds its way into the boiling tubes will dissolve in the toluene and give undesirable colored solutions. Marking with a lead pencil on a ground glass spot on these tubes is permissible.

Blood: $\frac{\gamma \text{ G. A.} \times 10 \times 100}{3 \times 1000}$ = milligrams glucuronic acid per 100 cc.

Urine: $\frac{\gamma \text{ G. A.} \times 50}{3 \times 1000}$ = milligrams glucuronic acid per cc. urine, when 50 is the dilution factor of the urine.

*Light absorption curves of the naphthoresorcinol reaction product.* Curves (Figure 2) for protein-free filtrates of human blood, urine, and a sample of pure glucuronolactone are shown in which the light absorption of the purple toluene layer was measured in a Beckman model B spectrophotometer over the range 400–600 mμ.

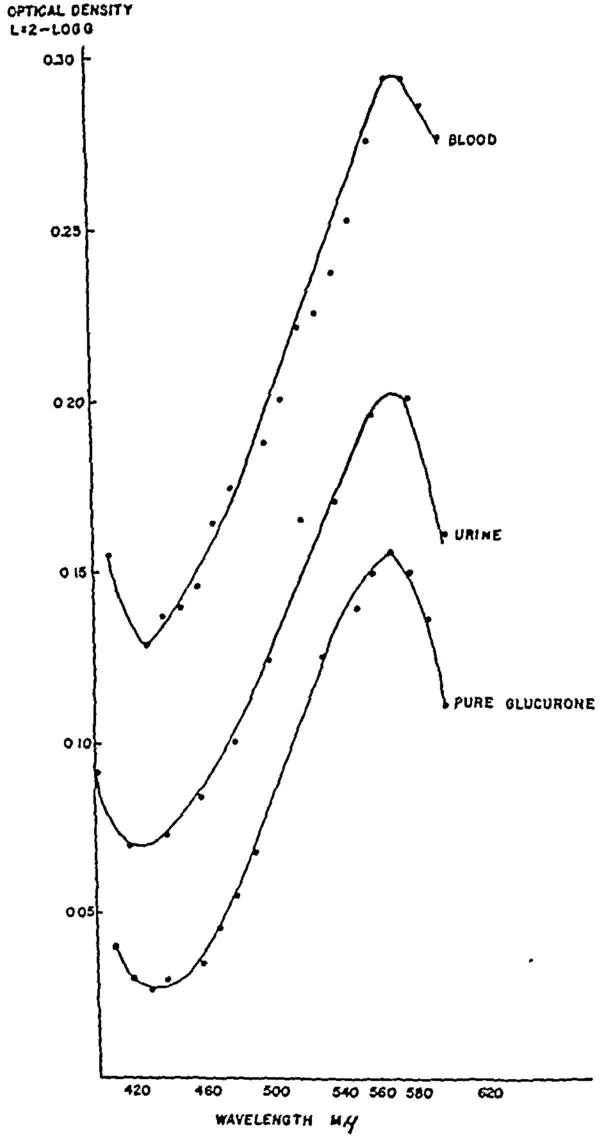

FIG. 2. LIGHT ABSORPTION CURVES OF THE TOLUENE SOLUTIONS OF THE GLUCURONIC ACID-NAPHTHORESORCINOL PRODUCT OBTAINED FROM PURE GLUCURONE (20 γ), A PROTEIN-FREE BLOOD FILTRATE (49 γ), AND URINE, DILUTED 1 TO 50 (30 γ)

TABLE I

*Recovery of glucuronic acid added to blood and urine*

| Experiment number | Glucuronic acid | Glucuronic acid added | Total glucuronic acid | |
|---|---|---|---|---|
| | | | Found | Calculated |
| | *mg./100 cc.* | *mg./100 cc.* | *mg./100 cc.* | *mg./100 cc.* |
| *Blood* | | | | |
| 1. | 5.4 | 3.5 | 9.6 | 8.9 |
| | 5.4 | 7.0 | 11.2 | 12.4 |
| 2. | 6.2 | 3.5 | 9.5 | 9.7 |
| | 6.2 | 7.0 | 13.0 | 13.2 |
| 3. | 6.0 | 3.5 | 8.3 | 9.5 |
| | 6.0 | 7.0 | 10.8 | 13.0 |
| 4. | 7.6 | 3.5 | 10.8 | 11.1 |
| | 7.6 | 7.0 | 14.2 | 14.6 |
| 5. | 6.7 | 3.5 | 10.0 | 10.2 |
| | 6.7 | 7.0 | 12.3 | 13.7 |
| *Urine* | | | | |
| 1. | 30.4 | 8.4 | 36.4 | 38.8 |
| | 30.4 | 16.8 | 49.5 | 47.2 |
| 2. | 54.0 | 8.4 | 61.6 | 62.4 |
| | 54.0 | 16.8 | 79.1 | 70.8 |
| 3. | 37.6 | 8.4 | 45.0 | 46.0 |
| | 37.6 | 16.8 | 50.8 | 54.4 |
| 4. | 54.1 | 8.4 | 58.3 | 62.5 |
| | 54.1 | 16.8 | 68.0 | 70.9 |
| 5. | 45.2 | 8.4 | 49.5 | 53.6 |
| | 45.2 | 16.8 | 60.6 | 62.0 |
| 6. | 36.6 | 16.8 | 48.3 | 53.4 |
| | 34.1 | 16.8 | 46.3 | 50.9 |
| | 56.7 | 16.8 | 70.0 | 73.5 |

*Recovery of glucuronic acid added to blood and urine.* In Table I are listed the recoveries of pure glucuronic acid added to whole blood and urine. In the upper range of blood glucuronic acid levels (8.9–11.1), the standard error was 7.3 per cent (range − 7 to + 14) and for all the recovery experiments, the standard error was 8 per cent (range − 20 to + 14). This performance of the method is similar to that of the method of Ratish and Bullowa (10).

*Distribution of blood glucuronic acid values in health and in some disease states.* In Figure 3 is illustrated the distribution of blood glucuronic acid in 100 normal individuals and in two groups of patients, one with arthritis and the other with cancer

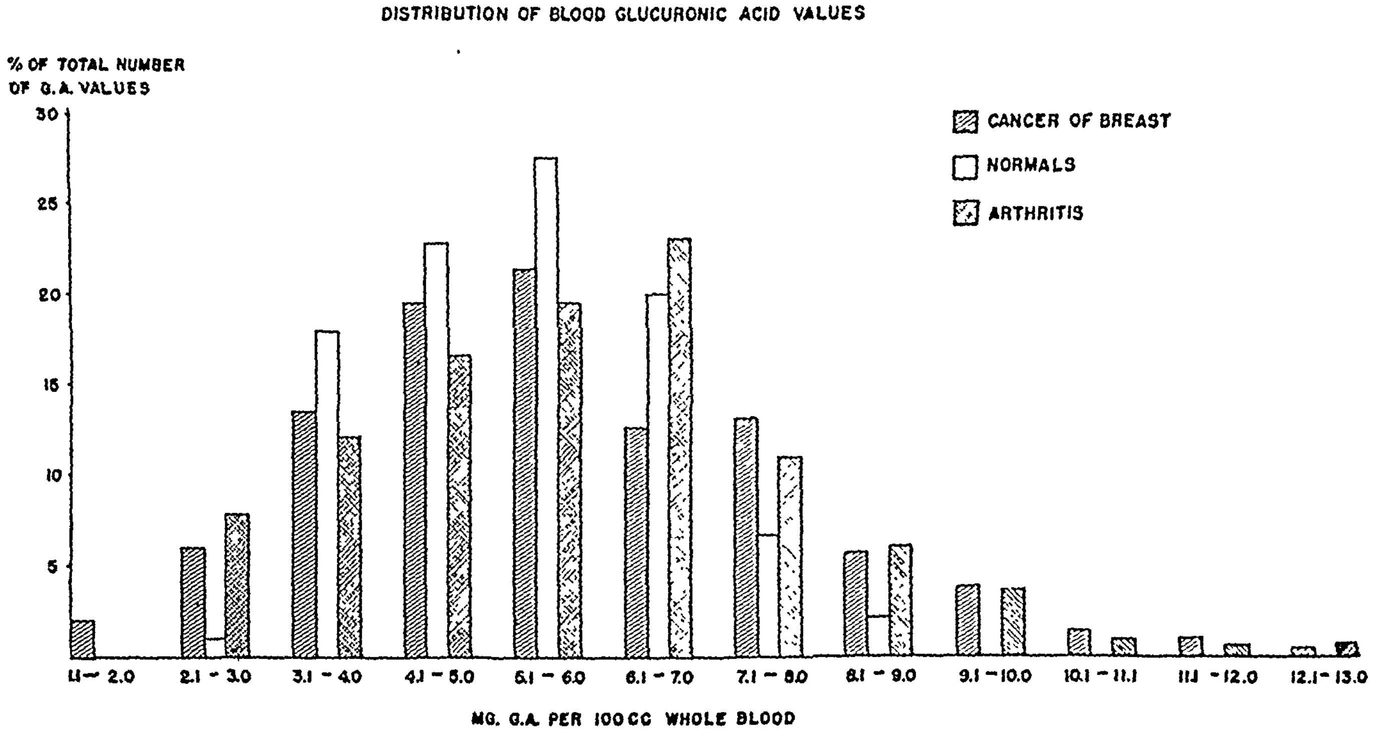

FIG. 3. DISTRIBUTION OF GLUCURONIC ACID (G.A.) VALUES IN NORMAL HEALTHY INDIVIDUALS (100), IN CANCER PATIENTS (128), AND IN ARTHRITIC SUBJECTS (44).

of the breast. In Table II are presented additional data on blood glucuronic acid in disease states. Values for blood β-glucuronidase activity obtained in these same patients have been included.

## DISCUSSION

It would appear that the majority of human blood glucuronic acid values range from 4 to 8 mg. per 100 cc., averaging 6 mg. per 100 cc. These values are higher than those reported by Laing and Anderson (13) (2.3–6.6 mg. per 100 cc., average 4.0), and include values lower than those obtained by Ratish and Bullowa (10) (6.8–9.2 mg. per 100 cc., average 7.5). Ratish and Bullowa state that results out of accuracy range are obtained with amounts of glucurone 5 mg. or less per 100 cc. The procedure of Laing and Anderson is similar to that of Ratish and Bullowa, with the greater possibility of incomplete color development due to a shorter heating period and the omission of alcohol in the process of pigment extraction. However, under the experimental conditions described here there is a greater opportunity for maximum pigment formation (one hour heating) and

TABLE II

*Concentration of blood glucuronic acid and serum β-glucuronidase in health and disease*

| Diagnosis | Glucuronic acid | | | | β-Glucuronidase | | | |
|---|---|---|---|---|---|---|---|---|
| | Number of patients | Number of determinations | Range of values | Average value (*mg./100 cc. blood*) | Number of patients | Number of determinations | Range of values | Average value (*units/100 cc. serum*) |
| No disease | 100 | 104 | 3.0–9.0 | 6.1 | 230 | 500 | 41–1285 | 389 |
| Cancer (all types) | 128 | 156 | 3.0–19.4 | 7.5 | 143 | 428 | 100–2340 | 587 |
| Cancer of breast | 57 | 90 | 3.0–9.0 | 5.9 | 57 | 280 | 114–2340 | 638 |
| Cancer of cervix | 5 | 13 | 4.4–8.4 | 6.7 | 19 | 27 | 206–1210 | 544 |
| Cancer of lung | 5 | 6 | 4.7–8.1 | 6.4 | 11 | 16 | 329–1360 | 718 |
| Cancer of intestinal tract | 6 | 16 | 3.7–14.0 | 6.9 | 20 | 39 | 191–1945 | 514 |
| Heart disease | 33 | 36 | 1.8–10.1 | 5.1 | 40 | 43 | 141–1093 | 439 |
| Diabetes | 5 | 11 | 2.3–8.1 | 5.1 | 5 | 21 | 181–1320 | 447 |
| Pregnancy | 15 | 15 | 4.0–9.7 | 6.2 | 15 | 15 | 262–1395 | 798 |
| Toxemia of pregnancy | 6 | 6 | 5.3–9.5 | 6.8 | 5 | 5 | 675–1560 | 1151 |
| Arthritis (male) | 7 | 94 | 2.2–11.3 | 5.5 | 7 | 91 | 247–1090 | 552 |
| Arthritis (female) | 37 | 191 | 2.0–12.0 | 5.8 | 39 | 208 | 141–1395 | 468 |

for efficient extraction of the resulting pigment (solution of the pigment first in alcohol and subsequent extraction with toluene). We have not encountered any interference of urinary pigment mentioned by Maughan and associates (11) in the determination of glucuronic acid in the urine.

From an inspection of the light absorption spectra of the toluene extract obtained by processing human blood and urine, it seems that under ordinary circumstances only the pigment produced by the glucuronic acid-naphthoresorcinol reaction is present and has an absorption maximum at 570–580 mμ. Pure glucurone solutions yield toluene solutions of pigment absorbing at 570 mμ. Ratish and Bullowa have shown that interference due to pentoses and glucose is negligible. Maughan and associates have likewise found the naphthoresorcinol reaction to be negative for many body metabolites. As mentioned before, galacturonic acid reacts with naphthoresorcinol.

The experience in this laboratory indicates an error in the recovery experiments of approximately 8 per cent. Much of this is due to the fact that analyses are rejected only if duplicate tubes differ by more than 2 galvanometer divisions in their readings of transmittance. This is the outside limit within which duplicate determinations of most other substances, *e.g.*, blood glucose, will agree when color measurements are made with the Evelyn photoelectric colorimeter. Accordingly, greater accuracy can be obtained either by the use of triplicate determinations, or by the development of conditions which will increase still further the optical density of the final toluene extract. For our purposes, the present procedure was found satisfactory.

The comparison of glucuronic acid values in the blood of healthy and diseased individuals is incomplete. It is clear, however, that in certain cases of arthritis and cancer there is a tendency toward abnormal values out of the range of the normal subjects studied. No clear correlations can be made between serum β-glucuronidase activity and blood glucuronic acid in the diseases studied.

## SECTION II. GLUCURONIC ACID TOLERANCE CURVES IN HUMANS

When studying the metabolism of any substance, it has been found useful to obtain some measure of the degree of utilization of the substance by the body. Accordingly, it was considered worthwhile to develop a tolerance test for glucuronic acid in humans in order to study the utilization of glucuronic acid and factors which may influence its metabolism.

Employing the method for blood glucuronic acid described in the first section of this paper, blood levels of glucuronic acid were determined before, and at intervals after, oral administration of glucuronolactone. It was found that only a small proportion of this substance was excreted in the urine.

Glucuronic acid tolerance curves were then obtained on 17 normal subjects, on 12 patients with arthritis and on 10 patients with various types of cancer. A description of the technique for performing the glucuronic acid tolerance curve and the results obtained on various human subjects are presented in this section.

TABLE III

*Urinary excretion of glucuronic acid before, during and after performance of the glucurone tolerance test*

The excretion of glucuronic acid in a single subject on five consecutive days gave the following 24 hour values: 0.64, 0.63, 0.58, 0.75, 0.31 grams.

| Patient | Clinical diagnosis | Urinary glucuronic acid (*grams/24 hours*) | | |
|---|---|---|---|---|
| | | Day prior to tolerance test | Day of tolerance test | Day after tolerance test |
| L. I. | Rheumatoid arthritis | 0.82 | 1.62 | 0.49 |
| O. B. | Carcinoma of lung | 0.50 | 0.39 | 0.39 |
| C. F. | Carcinoma of bladder | 0.67 | 0.55 | 1.04 |
| O. S. | Toxemia of pregnancy | 0.83 | 0.53 | 0.64 |
| E. C. | Rheumatoid arthritis | 0.23 | 0.56 | 0.32 |
| K. H. | Myasthenia gravis, arthritis | 0.34 | 1.02 | 0.44 |
| K. J. | Rheumatoid arthritis | 0.65 | 1.44 | 0.65 |
| M. E. | Carcinoma of breast | — | 0.91 | 0.52 |
| G. A. | Arthritis | 0.28 | 0.72 | 0.23 |

*The glucuronic acid tolerance curve.* Five cc. of venous blood are taken from the antecubital vein from a fasting subject immediately before the oral ingestion of 5 grams of glucurone[5] dissolved in 100 cc. $H_2O$. The same amount of blood is drawn at 30 minutes, and one, two, three, six and 24 hour intervals thereafter. The blood is collected in an oxalated test tube to prevent clotting. Folin-Wu filtrates are then prepared from 2 cc. of this blood and

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[5] Glucurone is an abbreviation for glucuronolactone. In the body glucurone is undoubtedly in equilibrium with glucuronic acid, thus: glucuronolactone $+ H_2O \rightleftarrows$ glucuronic acid. Pure glucurone was supplied through the courtesy of the Commercial Solvents Corporation, New York.

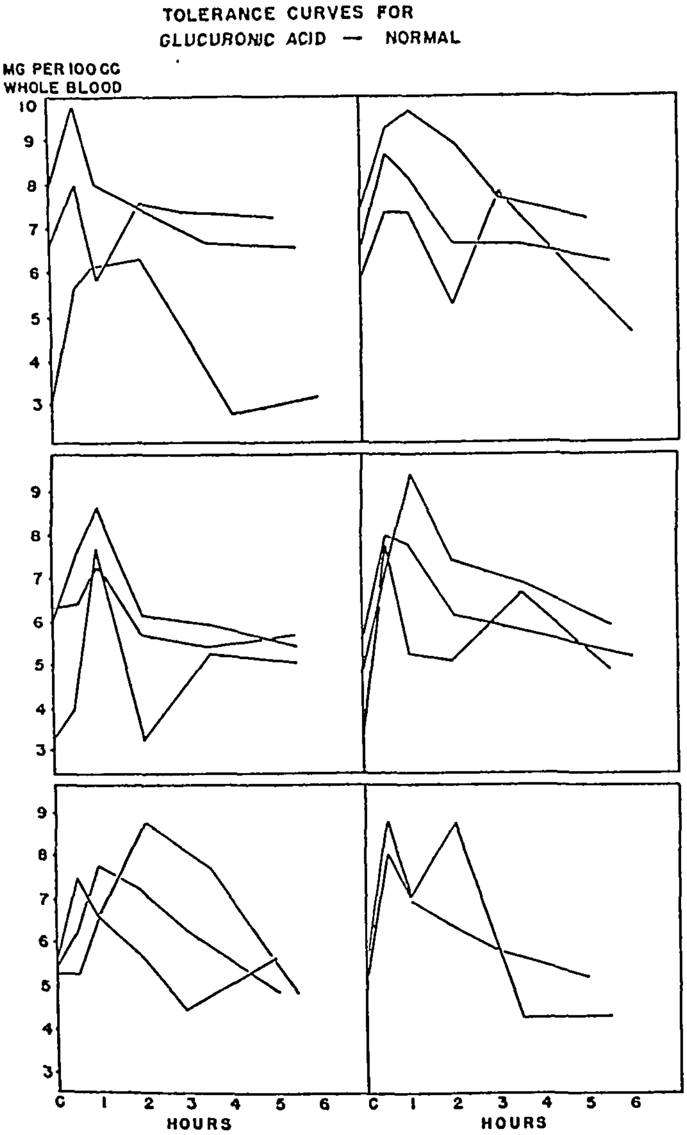

FIG. 4. GLUCURONIC ACID TOLERANCE TESTS IN NORMAL HEALTHY INDIVIDUALS

the glucuronic acid determined as described. These values are then related to time, as illustrated in Figures 4 and 5. These represent, respectively, individual tolerance curves and a composite graph, based on the individual data.

In some of these experiments 24 hour urine collections were made on the day before, the day of, and the day after performing the glucurone tolerance test. These data appear in Table III. For the present, an abnormal curve is defined as one which either (*a*) fails to show an elevation in blood glucuronic acid after glucurone ingestion (flat curve); (*b*) shows the glucuronic acid level, after reaching a peak, failing to fall by six hours to the pre-ingestion level (retenton curve); or (*c*) shows a fall of glucuronic acid concentration at six hours to a value much lower than the fasting level (declining curve).

In all of the tolerance tests, β-glucuronidase activity of blood serum was determined simultaneously (14) and a typical result, illustrating the behavior of blood glucuronic acid and β-glucuronidase activity, is shown in Figure 6.

## RESULTS

All of the normal subjects (Figure 4) exhibited an elevation in blood glucuronic acid following the ingestion of 5 grams of glucurone which appeared within 30 to 60 minutes and which persisted for as long as four hours. However, by six hours, in the majority of cases, the blood glucuronic acid levels had returned to the pre-ingestion level. In five subjects, two peaks in the curve were noted. A composite curve of these data is shown in Figure 5.

*Arthritis* (Figure 7). Five of 12 arthritic patients showed abnormal curves in which the level at six hours was as high as or higher than the peak elevation; the curves in the remainder appeared essentially normal.

*Cancer* (Figure 8). Four patients with carcinoma of the rectum exhibited abnormal, flat or retention curves. Two subjects with cancer of the lung, and one with cancer of the thyroid showed accentuated declines in their blood glucuronic acid levels after the peak had been reached. One patient with cancer of the lung at six hours still showed an elevation in blood glucuronic acid.

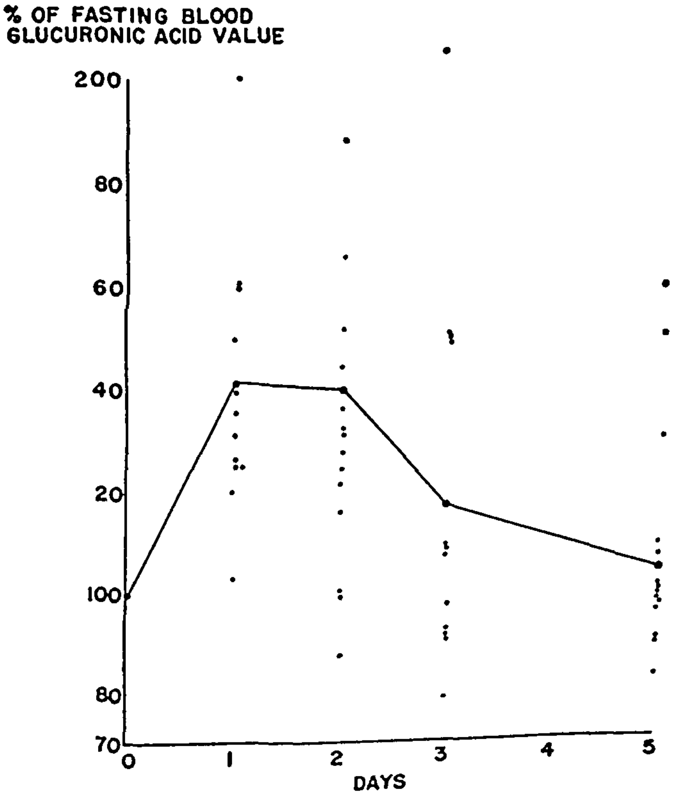

FIG. 5. A COMPOSITE CURVE OF THE NORMAL GLUCURONIC ACID TOLERANCE TESTS

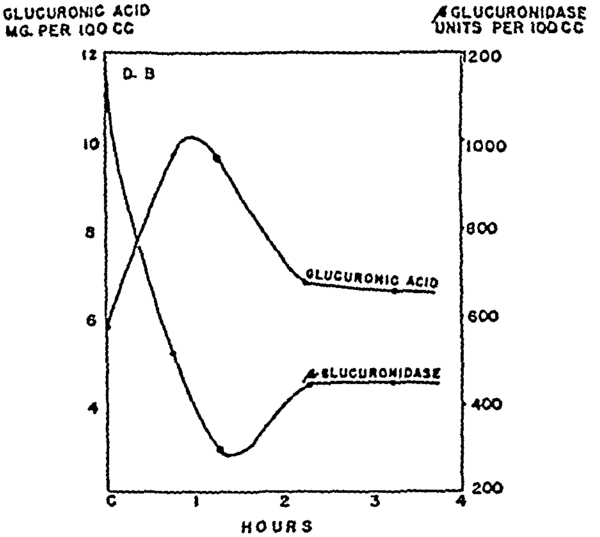

FIG. 6. INTERRELATION OF BLOOD GLUCURONIC ACID AND β-GLUCURONIDASE AFTER INGESTION OF GLUCURONIC ACID

Three of the cancer subjects had fasting levels of glucuronic acid above 8 mg. per 100 cc. of blood.

*Relation between blood glucuronic acid and serum β-glucuronidase.* Figure 6 shows the blood β-glucuronidase activity being reduced markedly at the same time when the blood glucuronic acid increases. After two hours, the values for both level off, that for glucuronic acid returns towards the pre-ingestion value and that for β-glucuronidase tends to remain far below the initial one. In all subjects studied, healthy as well as diseased, the blood β-glucuronidase was reduced following glucuronolactone ingestion. The extent of this effect and its persistence was variable, usually bearing some relationship to the fasting blood β-glucuronidase. Thus, the decline was most pronounced in subjects exhibiting initial β-glucuronidase activities above 500 units per 100 cc.

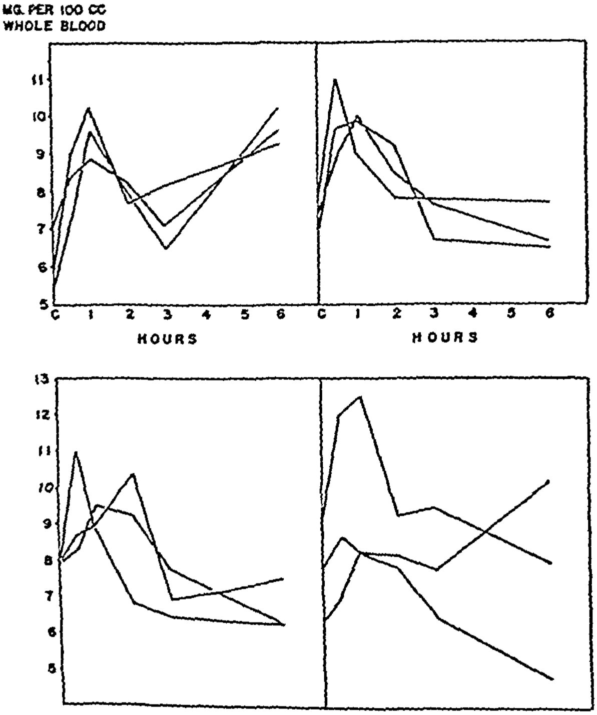

FIG. 7. GLUCURONIC ACID TOLERANCE TESTS IN ARTHRITIS

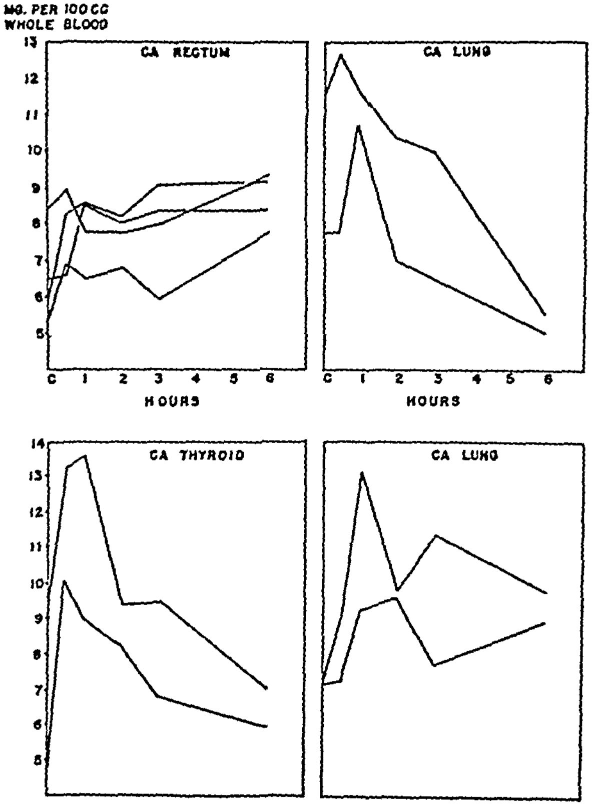

FIG. 8. GLUCURONIC ACID TOLERANCE TESTS IN CANCER PATIENTS

## DISCUSSION

The following possibilities suggest themselves to explain the reduction of β-glucuronidase activity in blood in the presence of an increased blood glucuronic acid: *1*) glucuronic acid may be inhibiting the activity of the enzyme, *2*) glucuronic acid may cause a shift of the enzyme to the formed elements of the blood (in which case the serum en-

TABLE IV

*Effect of the presence of added glucuronic acid on β-glucuronidase activity of serum and plasma*

The serum employed was prepared from blood taken from patients with cancer of the breast undergoing hormonal therapy which explains the higher β-glucuronidase activity observed as compared to the plasma enzyme activities (normal healthy subjects).

| β-Glucuronidase activity | Units/100 cc. |
|---|---|
| no glucuronic acid added | glucuronic acid added (*4 mg./100 cc.*) |
| *Serum* | |
| 1690 | 1630 |
| 930 | 890 |
| 673 | 673 |
| 683 | 695 |
| 930 | 905 |
| 943 | 960 |
| 900 | 900 |
| *Plasma separated from whole blood* | |
| 447 | 435 |
| 460 | 433 |
| 504 | 504 |
| 676 | 714 |

zyme would be depleted) or *3*) the glucuronic acid may cause blood β-glucuronidase to be removed from the blood for some as yet unknown purpose. As indicated in Table IV, there is no inhibition of serum β-glucuronidase in experiments where glucuronic acid is added *in vitro* in amounts corresponding to the highest concentrations of this substance observed in our tolerance curves. It is also clear that glucuronic acid added to whole blood does not result in a shift of β-glucuronidase from the plasma to the cells.

Since little change is found in the amounts of glucurone excreted in the urine during and after the tolerance test, there seems to be little doubt that the glucurone administered orally is utilized by the human subject. It remains to be determined which organ(s) take up this glucuronic acid and in which processes of metabolism the glucurone participates.

A pattern of glucuronic acid curves is evolving with three distinct types of curves. Some of these curves appear to be characteristic of certain types of cancer and to a much lesser extent arthritis and other chronic diseases (15).

## SECTION III. OBSERVATIONS IN PATIENTS RECEIVING ANTI-ARTHRITIC AND ANTI-TUMORAL AGENTS

### *Arthritis*

Four patients with an established diagnosis of rheumatoid arthritis were treated with ACTH, cortisone and glucurone and were studied from 70 to 150 days. All of these subjects at one or more periods in the study exhibited sudden and marked elevations in serum β-glucuronidase and in three, the blood glucuronic acid levels fluctuated markedly. The behavior of these two blood constituents in one of these patients whose arthritis was difficult to control is illustrated in Figure 9. In normal healthy subjects, blood glucuronic acid and serum β-glucuronidase levels are considerably more constant.

### *Cancer of the breast*

*A. Methylandrostenediol.* Thirteen patients with metastatic cancer of the breast were treated for two to 18 months with methylandrostenediol, a weakly-virilizing protein anabolic steroid (16). In three of these subjects, showing neither subjective nor objective clinical improvement, β-glucuronidase and glucuronic acid values showed no change during the course of therapy. In 10 patients with subjective improvement, including three with objective responses, the values of these blood constituents were abnormally high at one or more times during therapy. The behavior of blood glucuronic acid and serum β-glucuronidase levels is illustrated in one of these subjects in Figure 10. Clinically, there appeared to be a correlation of the increased levels of either one or both of these blood constituents with the improvement observed in the patient.

*B. Stilbestrol.* The administration of this hormone was found to increase the blood β-glucuronidase not only in women with cancer of the breast (17) but also in non-cancerous subjects (15). It had been found previously (18) that stilbestrol administered for 10 days of the post-partum period will prevent to a great extent the fall in serum β-glucuronidase which otherwise normally occurs following parturition. The resemblance of the effects of methylandrostenediol and stilbestrol upon the β-glucuronidase and the glucuronic acid of the blood is of interest from two points of view. First, it offers an avenue of approach to the development of chemical indices in the blood for evaluating the effect of chemo-therapeutic drugs in cancer of the breast and secondly, it suggests that in these subjects methylandrostenediol has a biochemical effect in common with stilbestrol.

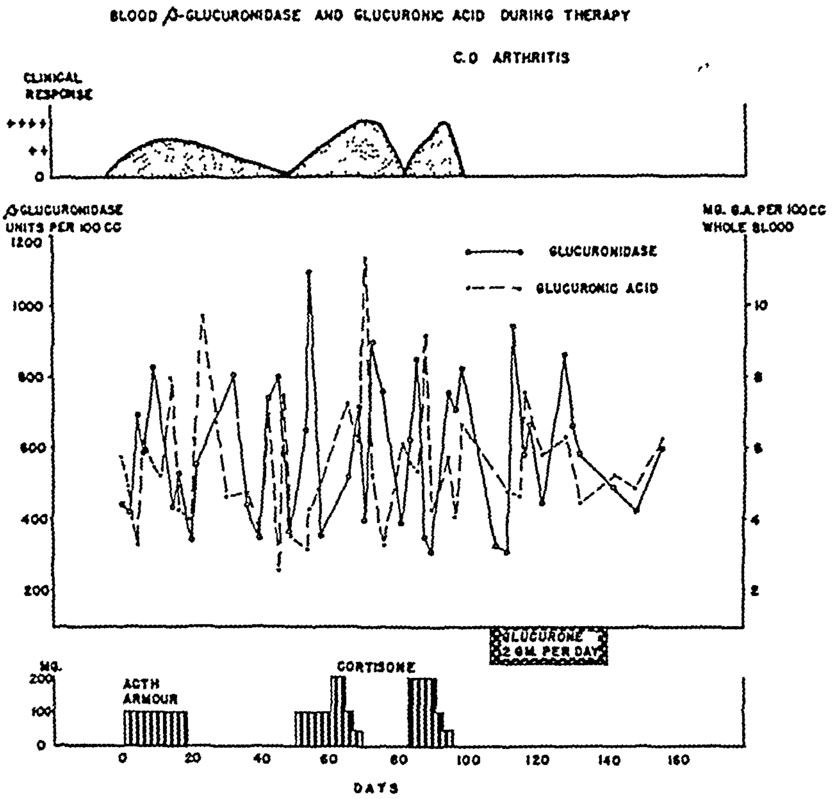

FIG. 9. VARIATIONS IN THE BLOOD GLUCURONIC ACID (G A) CONCENTRATION AND IN SERUM β-GLUCURONIDASE ACTIVITY IN AN ARTHRITIC PATIENT RECEIVING THERAPY

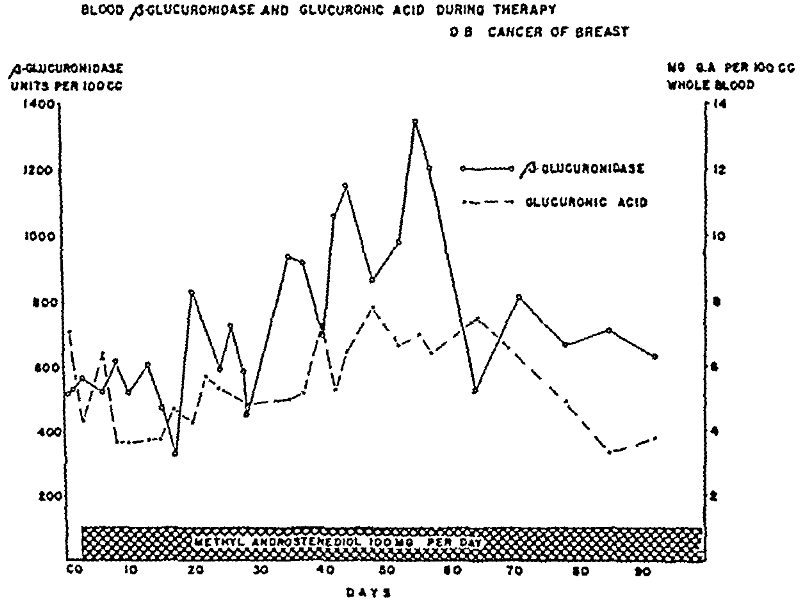

FIG. 10. THE EFFECT OF METHYLANDROSTENEDIOL ON BLOOD GLUCURONIC CONCENTRATION AND SERUM β-GLUCURONIDASE ACTIVITY

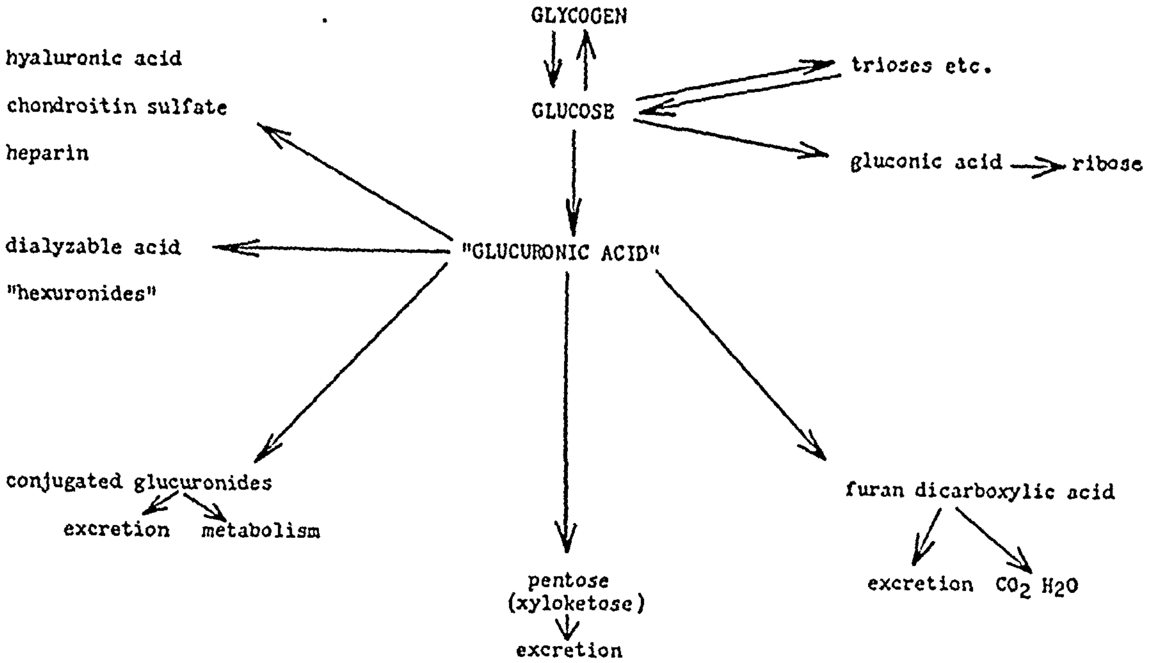

FIG. 11. A TENTATIVE SCHEME FOR GLUCURONIC ACID METABOLISM

It remains to be established whether the phenomena observed with regard to glucuronic acid and glucuronidase in the blood of patients with arthritis or cancer under therapy are specific for these disorders or whether they may be the reflection of a fundamental systemic effect without specific significance.

## GENERAL DISCUSSION

There are only a few known facts concerning the origin and fate of glucuronic acid in mammals.

*Origin of glucuronic acid.* It is generally believed that liver glycogen is the ultimate source of glucuronic acid. Quick (19), in 1926, showed that the administration of insulin to diabetic dogs greatly increased the output of glucuronic acid, while Kobayashi (20), in 1934, found low glycogen content in the livers of rabbits which had been given the glucuronidogenic drug, avertin. Later, Schmid (21) reported that the tolerance to menthol was much less in hibernating frogs whose liver glycogen had been depleted and Dziewiatkowski and Lewis (22) found a 50 per cent reduction in liver glycogen four hours after administering menthol to rats.

Direct evidence that glucose can be used for the synthesis of glucuronic acid in conjugated form was reported by Mosbach and King (23). After administering glucose labelled with $C^{14}$ uniformly in all six carbons to guinea pigs which were simultaneously given borneol, the glucuronic acid of the Zn bornyl glucuronide isolated from the urine was found to contain radioactive carbon, likewise uniformly distributed amongst its six carbon atoms.

On the other hand, administered glucuronic acid is not converted into glucose, since, to take one example, Quick (24) was unable to relieve insulin convulsions in mice by the administration of glucurone.

*Fate of glucuronic acid.* Glucuronic acid in amounts of 3 to 10 grams is almost completely metabolized by humans (24–26) and rabbits and dogs similarly metabolize glucuronic acid (27). Flaschenträger and colleagues (26) isolated furan dicarboxylic acid from the urine of healthy subjects who had received glucurone in amounts which indicated a 20 per cent conversion of the glucurone to this compound. The furan dicarboxylic acid itself is almost completely metabolized when given to humans.

Enklewitz and Lasker (25) gave glucurone to pentosuriacs, since they had seen increased pentosuria following the administration to these patients of glucuronidogenic drugs. Over 20 per cent of glucurone was apparently converted into the *l*-xyloketose which was identified. This effect was not seen following the administration of conjugated glucuronic acid.

Quick (24) first reported that dogs metabolized menthol and menthol glucuronide almost completely, and borneol and its glucuronide to a great extent. Smith and Williams (28) demonstrated that the rabbit metabolized stilbestrol glucuronide. It therefore seems reasonable that the intact conjugated glucuronides may undergo metabolism.

*Scheme of glucuronic acid metabolism.* In the accompanying scheme (Figure 11) the facts detailed above have been related. The evidence for dialyzable acid hexuronides rests on observations we have made on the nature of tissue uronides which show clearly that the dialyzable uronide of tissue is of a molecular species lower than hyaluronic acid and higher than that of the simple conjugated glucuronides. In all the pathways postulated uncertainty is indicated as to the exact mechanism. Final conclusive proof that glucuronic acid behaves in the manner outlined must await definitive studies, possibly utilizing glucuronic acid labelled with $C^{14}$.

*Present results.* In our studies, it has been found that glucuronic acid in 5 gram amounts given orally is almost completely utilized as judged by the urinary excretion of glucuronic acid.

*Glucuronic acid tolerance curves.* Of interest is the rapidity with which blood glucuronic acid levels, once elevated by oral glucuronic acid, decline to normal values. It would seem that the concentration of this substance is under homeostatic control by agencies which at present are unknown. The blood sugar and phosphorus remain unchanged during the course of glucuronic acid absorption and utilization. Accordingly, the postulated homeostatic mechanism is probably unrelated to glucose metabolism and may be related to formation of pentose and furan dicarboxylic acid.

*Observations in arthritis and cancer.* In certain patients, *e.g.*, those with cancer of the rectum, there appears to be a definite retention of glucuronic acid in the blood, and in others (see Figure 8) there exists a tendency for a marked decline in blood glucuronic acid. In all patients, the serum glucuronidase declined after ingestion of glucuronic acid. On the basis of the present data, no explanation can be advanced for these phenomena, nor can these phenomena be considered peculiar only for cancer and arthritis.

The lability of glucuronic acid concentration in the blood of patients with rheumatoid arthritis is interesting. It is known that glucuronic acid compounds such as hyaluronic acid and chondroitin sulfate are important constituents of cartilage and the joint capsule. The present observations raise the question whether or not arthritis may be accompanied by a disorder of glucuronic acid metabolism.

The frequent elevations of glucuronic acid seen in the blood of patients with carcinoma of the breast receiving methylandrostenediol therapy is a new observation for which no explanation is proposed.

Finally, it is found that the continuation of study in this relatively new field of investigation may help in our attempts to understand the interrelationships in metabolism of the steroid hormones, glucuronic acid and β-glucuronidase.

## CONCLUSIONS

1. A method for determining glucuronic acid in blood and urine has been developed by modifying existing procedures and by substituting toluene for ether in the extraction of the violet pigment produced in the naphthoresorcinol reaction. Toluene solutions of the pigment absorbed light maximally at 570–580 mμ. A linear relationship was found between the optical density of toluene extracts prepared from the reaction of naphthoresorcinol with glucuronic acid in the range of concentrations employed ($5\gamma$ to $40\gamma$).

Values are reported for 100 normal individuals and in a number of arthritics and patients with cancer of the breast.

2. Glucuronic acid (glucurone) administered orally appears to be utilized inasmuch as little of it is excreted in the urine. In normal subjects, a peak in the blood glucuronic acid is found within 30–60 minutes after ingestion which may persist from one to four hours. By six hours afterwards the blood glucuronic acid returned to pre-ingestion levels.

3. In five out of 12 arthritics and seven of 10 patients with cancer, these glucuronic acid tolerance curves were abnormal. Some showed what appeared to be retention of glucuronic acid in the blood while others were characterized by an unusually marked fall in glucuronic acid after the peak concentration had been passed.

4. Serum β-glucuronidase activity fell simultaneously with the rise in blood glucuronic acid seen in the glucuronic acid tolerance test.

5. These findings have been discussed in relation to the known facts of glucuronic acid metabolism which have been received.

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# EFFECTIVENESS OF A NEW COMPOUND, BENEMID, IN ELEVATING SERUM PENICILLIN CONCENTRATIONS

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(Submitted for publication February 14, 1951; accepted April 9, 1951)

The rapid renal clearance of penicillin has made it difficult to maintain blood levels adequate to combat resistant organisms such as enterococci and many staphylococci. Diodrast (1), p-amino-hippurate (PAH) (2), benzoic acid (3), sodium benzoate (3, 4), and carinamide (5), have been found to block renal tubular excretion of penicillin with enhancement of blood levels. Of these compounds, only carinamide has had wiedspread clinical use (6–8). Its value has been limited because the large doses required make it unwieldy and often poorly tolerated (9). This disadvantage appears to have been overcome by the recent discovery of a new compound, Benemid (10),[1] which is apparently effective in low doses, and in preliminary studies has been found to have little toxicity (11). This report is based on measurements of penicillin blood levels in 74 patients treated with intramuscular penicillin G or its procaine salt.

## MATERIALS AND METHODS

The subjects studied were adult patients in the King County Hospital who were receiving penicillin for a wide variety of infections. Patients with renal disease or congestive heart failure were excluded. After control penicillin levels were obtained, Benemid was administered in doses of 0.5 gm. every six hours for at least 12 to 24 hours prior to the measurement of the post-Benemid serum levels. Diet, salt, and fluid intake were not controlled. The concentrations of penicillin in the serum were determined by a tube dilution method, using killing of a Group A hemolytic streptococcus as the endpoint (12).

## CALCULATIONS OF DATA OBTAINED

Two methods were used to compute the increase in the penicillin levels after Benemid. The first method consisted of determining the increase found in each individual patient, and from these figures the average *individual fold increase* was calculated. The second method consisted of averaging all of the pre- and post-Benemid penicillin levels before computing the fold increase.

*Average of individual fold increases:* For each patient the fold increase of the serum penicillin level after Benemid was determined by dividing the post-Benemid level by the pre-Benemid level. These individual fold increases were then averaged. In a few instances there was a decrease rather than an increase following Benemid, and these fold decreases were subtracted from the total of the increases before dividing by the total number of patients.

*Fold increase of the average levels:* Serum penicillin levels before and after Benemid were averaged separately. The fold increase of the average levels was then determined by dividing the post-Benemid average by the pre-Benemid average.

## RESULTS

The first group (Table I) consisted of 19 patients who were receiving 300,000 units of procaine penicillin once daily. Levels before and after Benemid were measured on one occasion in each patient at three, 12 and 24 hours following a penicillin injection. Of the 57 occasions on which concentrations were determined before and after Benemid, there was an increase in the penicillin

TABLE I

*Effect of Benemid on serum penicillin levels of 19 patients receiving 300,000 units of procaine penicillin once daily*

| Hours after penicillin injection: | 3 hrs. | 12 hrs. | 24 hrs. |
|---|---|---|---|
| Average serum level (units/cc.) before Benemid | 3.14 | 1.07 | 0.23 |
| Range | 0.5–10.0 | <0.04–5.0 | <0.04–5.0 |
| Average serum level (units/cc.) after Benemid | 6.34 | 2.14 | 0.66 |
| Range | 0.5–20.0 | <0.04–7.0 | <0.04–2.0 |
| Fold increase of average serum levels | 1.99 | 2.00 | 2.86 |
| Average of individual fold increases | 2.03 | 4.37 | 2.82 |
| Range | 5 fold decrease to 10 fold increase | 1 to 10 fold increase | 4 fold decrease to 10 fold increase |

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[1] Sharp and Dohme's trade name for p-(di-n-propylsulfamyl)-benzoic acid.

level following Benemid 39 times (68.4%). In the other 18 instances, the levels remained the same, or were lower following Benemid (eight times at three hours; five times each at 12 and 24 hours). The averages of the individual fold increases were 2.03, 4.37, and 2.82 at three, 12 and 24 hours respectively, and the fold increases of the average levels were 1.99, 2.00, and 2.86.

When the group was divided into patients over the age of 60 and those under the age of 60, those over 60 had uniformly higher levels before Benemid, and also greater and more consistent increases in the levels following Benemid.

The second group (Table II) consisted of 27 patients treated with 300,000 units of procaine penicillin every 12 hours. Levels were measured three and 12 hours following a penicillin injection. Of the 54 determinations which were made before and after Benemid, there was an increase of the penicillin level following Benemid on 50 occasions (92.6%). Three levels at the three hour interval were the same before and after Benemid, and in one patient the level was lower following Benemid. The averages of the individual fold increases were 2.62 and 3.08 at three and 12 hours respectively, and the fold increases of the average levels were 2.11 and 2.67.

It was again noted that patients over the age of 60 had higher levels before Benemid, and a greater increase in penicillin concentrations following Benemid.

The third group consisted of five patients treated with 600,000 units of procaine penicillin every eight hours. In each instance the level after Benemid exceeded the control level. The average levels at the end of the eight hour period were increased by Benemid from 3.2 units/cc. to 10.4 units/cc. The fold increase of the average levels and the average of the individual fold increases were both approximately 3.2.

TABLE II

*Effect of Benemid on serum penicillin levels of 27 patients treated with 300,000 units of procaine penicillin every 12 hours*

| Hours after penicillin injection: | 3 hrs. | 12 hrs. |
|---|---|---|
| Average serum level (units/cc.) before Benemid | 2.62 | 0.84 |
| Range | 0.2–7.0 | 0.04–2.0 |
| Average serum level (units/cc.) after Benemid | 5.54 | 2.24 |
| Range | 0.7–10.0 | 0.2–10.0 |
| Fold increase of the average serum levels | 2.11 | 2.67 |
| Average of individual fold increase | 2.62 | 3.08 |
| Range | 2 fold decrease to 7 fold increase | 1 to 10 fold increase |

TABLE III

*Effect of Benemid on serum penicillin levels of 19 patients treated with 100,000 units of crystalline penicillin G every three hours*

| | Three hour serum penicillin level |
|---|---|
| Average serum level (units/cc.) before Benemid | 0.48 |
| Range | <0.04–4.0 |
| Average serum level (units/cc.) after Benemid | 1.43 |
| Range | 0.07–10.0 |
| Fold increase of the average serum levels | 2.98 |
| Average of individual fold increases | 5.32 |
| Range | 1 to 10 fold increase |

The fourth group (Table III) consisted of 19 female patients (average age 29) from the Gynecology Service who were treated with 100,000 units of crystalline penicillin every three hours. Serum penicillin levels were measured three hours after one of the penicillin injections. The levels following Benemid were higher in 18 patients (94.6%), and in the 19th, the level was the same before and after Benemid. Four patients (22%) had measurable levels only following Benemid. The average level before Benemid was 0.48 units/cc.; the average level following Benemid was 1.43 units/cc. In this series the average of the individual fold increases (5.32) was appreciably larger than the fold increase of the average levels (2.98). The significance of this difference will be mentioned in the discussion.

The fifth group (Table IV) consisted of four patients who received 1,000,000 units of crystalline penicillin every two hours. Two had pneumococcic meningitis, one had staphylococcic meningitis, and in the fourth the diagnosis was non-paralytic poliomyelitis. Serum penicillin levels were measured two hours following an injection of penicillin. The average serum level was increased by Benemid from 21 units/cc. to 73 units/cc. In two of the patients spinal fluid levels were determined both before and after Benemid, and showed increases of 10 to 40 fold.

### TOXICITY

The toxicity of Benemid appears to be of low order. Most of the patients in this study received Benemid for only 48 to 72 hours, and in all instances the dosage was 2 gm. per day. However, five patients received Benemid for over two weeks. In none of the patients was there any evidence of systemic toxicity. Intolerance was limited to one patient who vomited the medication, and one patient who complained of dizziness. No abnormal urinary findings were detected during or after Benemid administration.

### DISCUSSION

The foregoing data clearly indicate that the oral administration of a new compound, Benemid, causes a significant elevation of serum penicillin levels. In the 139 instances in which a comparison was made, penicillin levels were 3.39 times higher after Benemid. This is comparable to the elevation obtained with carinamide, and is accomplished with a much smaller dose. The lower dosage requirement is probably largely responsible for the greater tolerance of Benemid, and will undoubtedly lead to a much wider general acceptance.

It should be noted that penicillin concentrations were not invariably higher after Benemid administration. The least favorable results from this standpoint were obtained with procaine penicillin (300,000 units) injected once daily, in which an elevation occurred in only 68% of the instances. When procaine penicillin was administered twice daily, elevations occurred in 92%, and with aqueous penicillin administered every three hours, enhancement was recorded in 95% of cases. The marked variability in absorption of penicillin, especially the procaine salt, undoubtedly accounts for these results, and it is not surprising that the greatest inconsistencies occurred when penicillin was injected only once daily.

TABLE IV

*Effect of Benemid on serum and cerebrospinal fluid levels of four patients treated with one million units of penicillin every two hours*

| Patient | Before Benemid penicillin levels (*units/cc.*) | | After Benemid penicillin levels (*units/cc.*) | | Fold Increase | |
|---|---|---|---|---|---|---|
| | Serum | CSF | Serum | CSF | Serum | CSF |
| B. L. | 10 | | 50 | 7.0 | | |
| | 10 | | 50 | 7.0 | | |
| | | | 70 | | 5.7 | |
| C. U. | 7 | | 40 | | | |
| | 10 | 0.2 | 40 | 4.0 | | 20.0 |
| | | | 70 | | | |
| | | | 40 | 2.0 | 5.6 | 10.0 |
| J. O. | 40 | | | | | |
| | 20 | | 70 | 0.05 | 2.3 | |
| H. A. | 50 | 0.05 | 100 | 2.00 | 3.0 | 40.0 |
| | | | 200 | | | |
| Average | 21 | 0.12 | 73 | 2.76 | 4.15 | 23.3 |

In the present report, the enhancement of penicillin levels has been expressed mathematically in two ways, as the average of the individual fold increases, and as the fold increase of the average levels. Disparities between these two figures occurred in all major groups studied, and are a reflection of the marked variability of penicillin levels obtained in different patients. In any one patient, the results are usually fairly constant from day to day, but even under these circumstances there is enough variability in absorption to produce marked discrepancies. A good example occurred in Group 4, in which the average of the individual fold increases (5.32) was considerably higher than the fold increase of the average levels (2.98). Here, the exclusion of only one patient, whose pre- and post-Benemid penicillin levels were 4 and 10 units respectively, would have so altered the results that with both methods of calculation the final figure would have been approximately 5.3. The average of the individual fold increases would appear to be the most valid method of expressing the results, since it indicates the increment in levels in each patient. This point has seemed worthy of emphasis since it is not universally realized that there is a great variability in penicillin levels from patient to patient.

The administration of Benemid will probably contribute little to penicillin therapy in the majority of infections caused by penicillin sensitive organisms. However, there are certain conditions in which it may prove of great value in bringing about a favorable response, and in reducing the cost of treatment. Subacute bacterial endocarditis, especially when caused by penicillin resistant organisms such as enterococci, is one of the diseases in which a great deal may be accomplished by Benemid administration. Large amounts of penicillin are required to maintain levels as high as 20 units/cc. With Benemid, such levels may be elevated to 40 or 80 units/cc. without increasing the dosage of penicillin. In some cases this will undoubtedly bring about effective therapy, and will at the same time minimize the mechanical and economic problems of massive penicillin therapy. Infections with other penicillin resistant organisms, such as staphylococci and certain of the gram negative bacilli, also fall into this category. In the patients who received Benemid in addition to 1,000,000 units of crystalline penicillin every two hours, the blood levels were never lower than 40 units/cc. Concentrations of this magnitude should be effective in the therapy of certain infections which are not ordinarily considered susceptible to penicillin.

The results obtained in the four patients with meningitis were of special interest. Spinal fluid penicillin concentrations were elevated 10 to 40 fold in patients treated by the intramuscular route alone. The clinical response in these patients was striking, especially in a 43 year old woman who was seriously ill with staphylococcal meningitis. Benemid given in conjunction with large intramuscular doses of penicillin would appear highly efficacious in treating meningitis due to gram positive bacteria.

Finally, Benemid may find an important place in conjunction with oral penicillin therapy. The effectiveness of Benemid in prolonging therapeutic concentrations of penicillin should make it possible to achieve results with smaller doses, or with administration at greater intervals.

## SUMMARY AND CONCLUSIONS

The effect of the oral administration of 0.5 gm. of Benemid every six hours on serum penicillin levels was measured in 74 patients. In 51 patients treated with procaine penicillin, there was enhancement of penicillin levels following Benemid in 81% of the instances, with an average fold increase of 2.87. In 23 patients treated with crystalline penicillin the levels were higher in 96% of the cases, with an average fold increase of 5.10. This three to five fold enhancement of penicillin levels should make the therapy of certain penicillin resistant infections considerably less expensive, and simpler from a mechanical standpoint.

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# JOINT TEMPERATURE MEASUREMENT IN THE EVALUATION OF ANTI-ARTHRITIC AGENTS[1, 2]

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(Submitted for publication February 23, 1951; accepted April 10, 1951)

Accurate evaluation of the improvement in rheumatoid arthritis during therapy has always been greatly hampered by the lack of any quantitative criteria. Clinicians have depended upon a summation of clinical observation and a lowering of the erythrocyte sedimentation rate to prove a lessening of arthritic activity (1). Such clinical observations as decrease in joint tenderness or swelling, or increase in range of motion of the affected joints are subject to great variation of interpretation, depending upon the experience and enthusiasm of the physician, and upon the usual fluctuations of the disease process itself. The patient is often so enthusiastic about any new form of therapy that he minimizes his joint tenderness, and puts forth extra effort in attempting motion. The erythrocyte sedimentation rate, for years the only laboratory index of degree of activity in rheumatoid arthritis, is a non-specific test. Decrease in the rate often lags far behind obvious clinical improvement, and the degree of elevation of the sedimentation rate is not always in proportion to the activity of the arthritis (2). The ideal yardstick for improvement must be objective, must show changes promptly when they occur; the gradient must be sufficient to overshadow technical errors, and it must be consistent under standard conditions.

In previous reports, Hollander and Horvath (3, 4) have shown that the internal temperature of the rheumatoid arthritic joint appears to be elevated in close correlation with the amount of local inflammation as estimated from careful clinical evaluation. The internal temperature of a chronically inflamed joint varies little from day to day, or even from week to week, under standard conditions of rest and room temperature except when distinct changes in activity of the disease occur (4). Application of heat or cold over a joint, exercise (4, 5), and administration of certain drugs produce significant changes in joint temperature (6). Because of these observations, particularly the fact that intra-articular temperature determination apparently measures objectively the degree of inflammation of the rheumatoid arthritic joint, it was decided to study the effect of various anti-arthritic agents on the joint temperature in rheumatoid arthritis.

## METHODS

As previously described (4), the method for determining intra-articular temperature consisted of inserting a filamentous copper-constantan thermocouple into the joint space (usually the knee) through the bore of an aspirating needle. In the experiments here reported, the e.m.f. developed was translated into degrees Centigrade by an automatic recording electronic potentiometer, which printed the temperature readings on a kymographic record. Determinations were made in a constant temperature room (22° ± 1° C.) with the patient at rest for 30 minutes.

At the time the aspirations for joint temperature determination were performed, specimens of the joint fluid were obtained for total leukocyte count. The synovial fluid specimens in oxalated tubes were examined within one half hour after collection. The fluid was diluted in a white cell pipette and counted in a standard counting chamber in the usual way. Tenth normal hydrochloric acid was used as the diluting fluid. Specimens which contained gross blood were not included.

Serial joint temperature determinations were made on 21 afebrile patients with active rheumatoid arthritis. In 19 of these an inflamed knee was studied, and in two the temperature in an inflamed ankle was measured. Control observations were made on two or three consecutive days prior to administration of cortisone acetate[6] or

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[1] Read in part before the annual meeting of the American Rheumatism Association, San Francisco, California, June 23, 1950.

[2] Aided in part by a grant from the United States Public Health Service.

[3] National Foundation for Infantile Paralysis, Fellow in Physical Medicine.

[4] Ayerst, McKenna and Harrison Fellow of the Association for the Study of Internal Secretions.

[5] Fellow of the Belgian-American Education Foundation.

[6] The cortisone acetate was supplied by Merck and Co. through Dr. James M. Carlisle, Medical Director, who also kindly supplied the tablets of cortisone used.

pituitary adrenocorticotropic hormone (ACTH)[7] to be sure the pretreatment temperature was consistent. Eleven patients were observed with parenterally administered cortisone, and ten with ACTH. The dose of cortisone was 300 mg. the first day, and 100 mg. daily thereafter. ACTH was administered in 80 mg. daily doses (20 mg. every six hours). Joint temperature determinations were made four hours after the first dose of drug in six instances, and daily determinations were made throughout the observation period in all.

In addition to the above studies, cortisone acetate tablets[6] were administered orally to six patients with rheumatoid arthritis and serial joint temperature studies were performed. Control temperatures were taken just previous to administration and again after 48 and 96 hours of therapy. The oral dosage of cortisone was 150 mg. daily, administered as three doses of 50 mg. each for the first three days of the study period. The amount was then reduced to 100 mg. daily in divided doses. No other medication was used during the period of study in any case.

As an additional experiment eight patients with rheumatoid arthritis were given various agents, supposedly "anti-arthritic," for a period of one week in recommended "effective" doses. Following this, and without the patient's knowledge, 100 mg. daily of cortisone were substituted for the unproved "anti-arthritic" agent being assayed. Daily joint temperature determinations were made throughout the experiment in each instance.

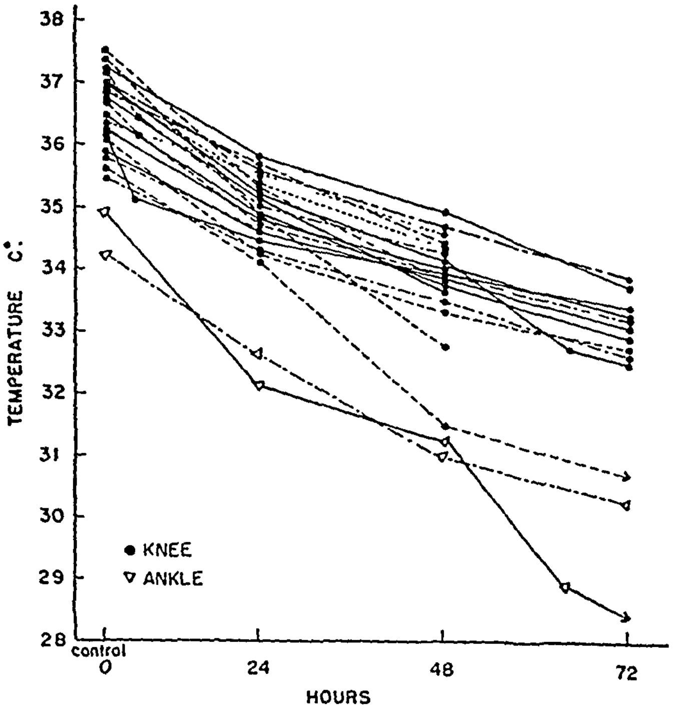

FIG. 1. THE INITIAL FALL IN INTRA-ARTICULAR TEMPERATURE IN 21 CASES OF RHEUMATOID ARTHRITIS ON CORTISONE OR ACTH

Slopes in the ten on ACTH and the 11 on cortisone were so similar that they are not differentiated.

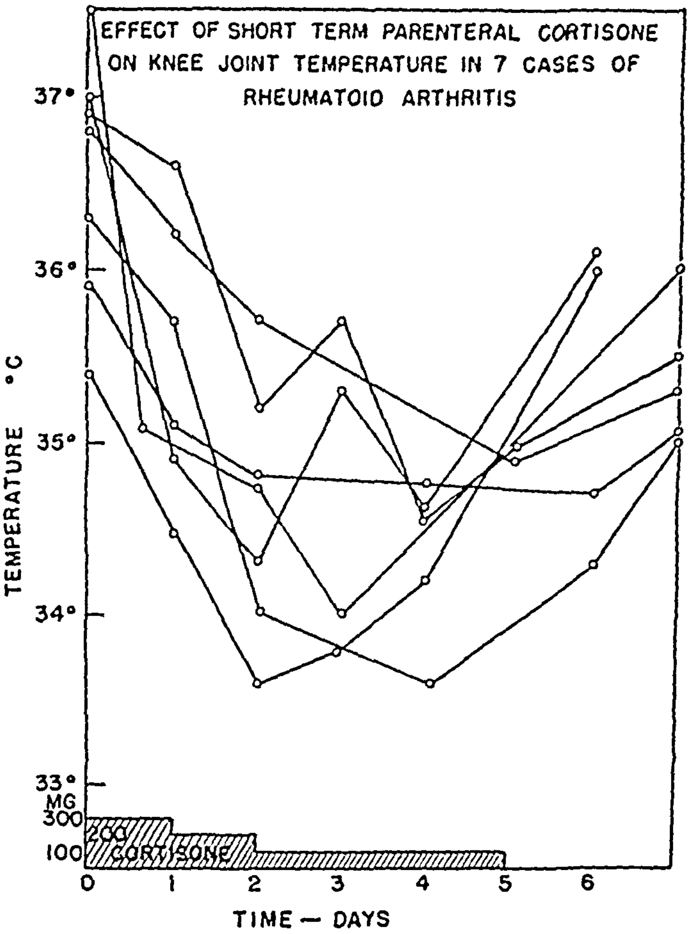

FIG. 2. TYPICAL VARIATIONS IN KNEE JOINT TEMPERATURE DURING AND AFTER ADMINISTRATION OF CORTISONE (OR ACTH)

## RESULTS

### *Effect of Parenteral ACTH and Cortisone*

Within 24 hours of the initiation of hormone therapy, a prompt and consistent fall in joint temperature in every case was demonstrated. This initial trend is illustrated by Figure 1. The minimum decrease in joint temperature during the first 24 hours of administration of cortisone or ACTH was 0.7° C., the maximum decrease was 2.7°, and the average was 1.4° C. This is in contrast to a maximum fluctuation of 0.4° during the control period. Since the rate of fall in both ACTH and cortisone treated cases was identical, they are not differentiated in the figure. On subsequent treatment days the joint temperature continued to decrease, usually approaching normal levels within three to five days (*i.e.,* approximately 33° C. for the knee, and 29° C. for the ankle). A careful statistical analysis of the figures showed that the rate of fall was significant.[8]

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[7] The ACTH was supplied by Organon, Inc., through Dr. Kenneth Thompson, Medical Director, and by Armour Laboratories, through Dr. John R. Mote, Medical Director.

It was observed that the fall in joint temperature on treatment with the hormones preceded detectable clinical improvement in 14 of the 21 patients, and preceded by some days any significant change in the sedimentation rate in all but two instances. In five of the patients a rebound, or temporary increase in the joint temperature, was noted between the third and fifth day of treatment. Characteristic curves of the joint temperature response during and after cortisone administration are charted on Figure 2. These same variations were seen with ACTH.

The joint temperature began to rise within 24 to 48 hours after stopping ACTH or cortisone administration in all cases studied. This rise preceded clinical relapse in 17 instances. In five patients, however, some rise in temperature of the joint was noted even before the hormones were stopped. In 14 instances the joint temperature had returned to pre-treatment levels within one week after cessation of cortisone or ACTH, but in seven cases (five ACTH treated, and two cortisone treated) the relapse to pre-treatment joint temperature levels was delayed until the ninth to 13th post-treatment day.

The serial synovial fluid cell counts on the patients during administration of cortisone or ACTH showed a pronounced decrease in leukocytes which paralleled the fall in joint temperature. This is illustrated in Figure 3. Frequent technical difficulties from clotting of the fluid, contamination with blood or insufficient fluid interfered with accurate results. Because of such difficulties only nine of the 21 cases under study could be charted.

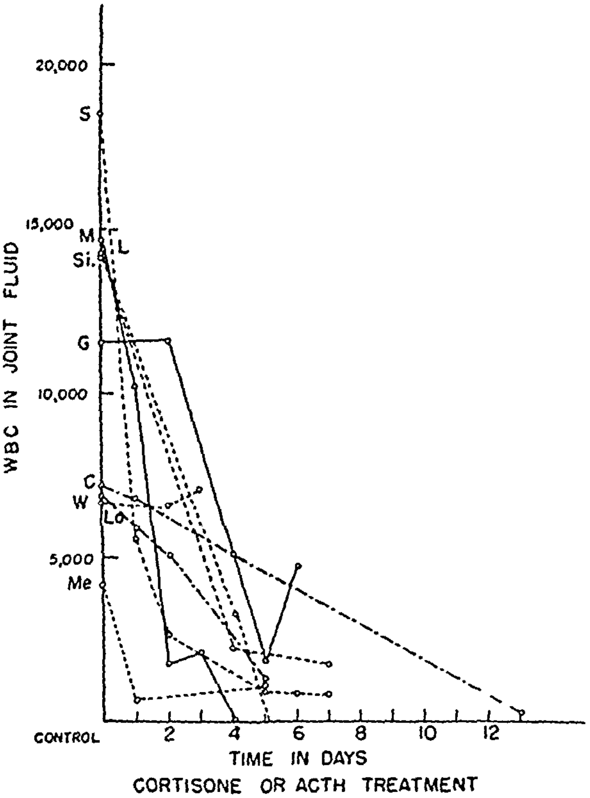

FIG. 3. THE EFFECT OF ACTH OR CORTISONE ADMINISTRATION ON THE JOINT FLUID CELL COUNT IN NINE CASES OF RHEUMATOID ARTHRITIS

Cases S, M, C, and La were on ACTH; L, Si, G, W, and Me were on cortisone.

### *The Effect of Oral Cortisone on Intra-articular Temperature*

In all six patients to whom cortisone acetate was administered orally in the form of tablets, a marked drop in joint temperature was noted within 48 hours. Since these were out-patients, an initial 24 hour joint temperature determination was not accomplished. Figure 4 illustrates the drop in joint temperature after oral administration which paralleled the fall in joint temperature seen on parenteral administration (Figure 1). The clinical improvement noted in these patients was comparable to that seen with parenteral administration of cortisone.

### *The Effect of Other So-called "Anti-arthritic" Agents versus Cortisone on Joint Temperature*

Since the method of joint temperature determination showed such a prompt change in *every* case treated with cortisone or ACTH, it has been used to test other possible "anti-arthritic" agents. Several steroids have been recommended for treating rheumatoid arthritis: delta-5-pregnenolone (7), testosterone (8), 21-acetoxypregnenolone (Artisone) (9), and others. Some of these were identified only by number as their structural formulae and chemical names had not been revealed at the time of study. The steroids used for the study

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[8] Acknowledgment is made to Dr. J. Harold Austin, Emeritus Professor of Research Medicine, University of Pennsylvania, for the statistical analysis of the data.

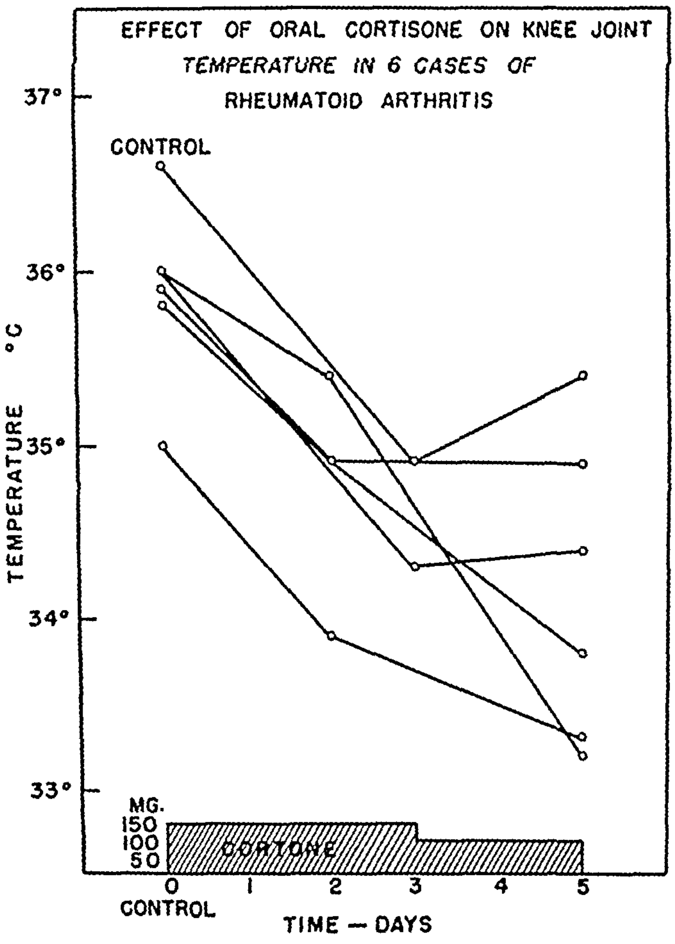

FIG. 4. THE INITIAL EFFECT OF ORALLY ADMINISTERED CORTISONE ACETATE ON KNEE JOINT TEMPERATURE IN SIX CASES OF RHEUMATOID ARTHRITIS

were (*a*) No. 10270 (16-dehydropregnenolone acetate), (*b*) 21-acetoxypregnenolone (Artisone), (*c*) testosterone propionate, (*d*) delta-5 pregnenolone, and (*e*) C.S. 117 (21-acetoxypregnenolone acetate).[9] In addition, one cinchoninic acid derivative (3-hydroxy, 2-phenyl cinchoninic acid), referred to as H.P.C., has been recommended for its possible anti-rheumatic activity (10). Because of the unproved effects of the "other steroids," as well as their unknown nature in some instances, they have been labelled in Figure 5 as "mysteroids." The doses of each agent used are given in the legend of Figure 5. Each one was administered in the "recommended" amounts, and continued for one week, after which cortisone was substituted in the usual dosage. Only one or two examples of treatment with each "mysteroid" were studied with serial joint temperature determination, as the clinical results in a series of patients treated with each of these other agents had been discouraging. The *contrasting effect, in each of the eight* cases, of the unproved drug and cortisone on the joint temperature of the inflamed knee is apparent. These patients had been admitted "for cortisone treatment," so believed they were receiving cortisone from the beginning of the study. None of the "mysteroids" produced any significant fall in joint temperature nor objective clinical improvement, yet in every case a prompt and marked fall occurred with the administration of cortisone. The fall in joint temperature with the start of cortisone administration was accompanied by prompt clinical improvement in every case studied. Interestingly enough, without exception, these patients, even though unaware that cortisone had been substituted, remarked on this sudden change in effectiveness. The joint temperature in the patient given H.P.C. orally was depressed about 1° C. from the control level throughout the

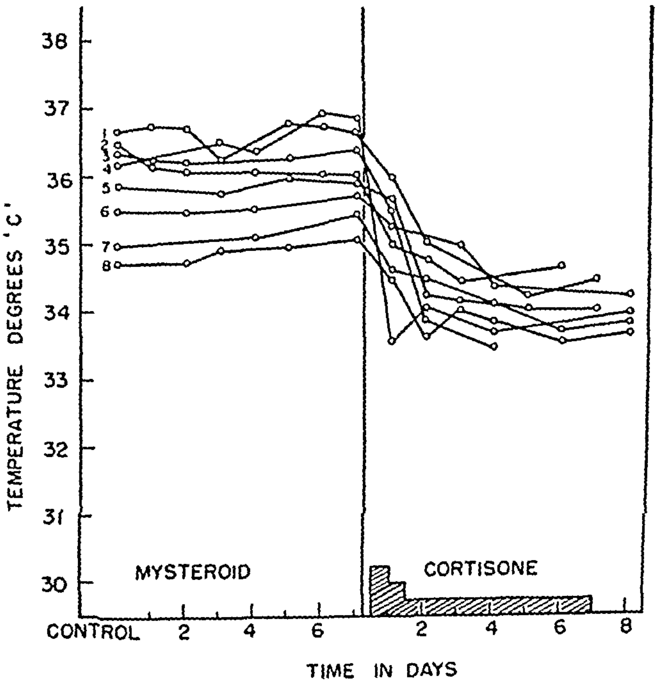

FIG. 5. THE COMPARATIVE EFFECT OF ADMINISTRATION OF OTHER "ANTI-ARTHRITIC" AGENTS (MYSTEROIDS) AND CORTISONE ON THE KNEE JOINT TEMPERATURE IN EIGHT CASES OF RHEUMATOID ARTHRITIS

Cases 1 and 4 received 300 mg. daily of 16-dehydropregnenolone; cases 3 and 7 received 300 mg. daily of 21-acetoxypregnenolone; case 2 received orally 40 mg./kg. body weight daily of H.P.C.; case 5 received 200 mg. of testosterone propionate daily; case 6 received 300 mg. daily of delta-5 pregnenolone, and case 8 received 300 mg. of 21-acetoxypregnenolone acetate daily during the initial period.

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[9] The above agents were kindly supplied by Eli Lilly and Co., Organon, Inc., and Wyeth, Inc.

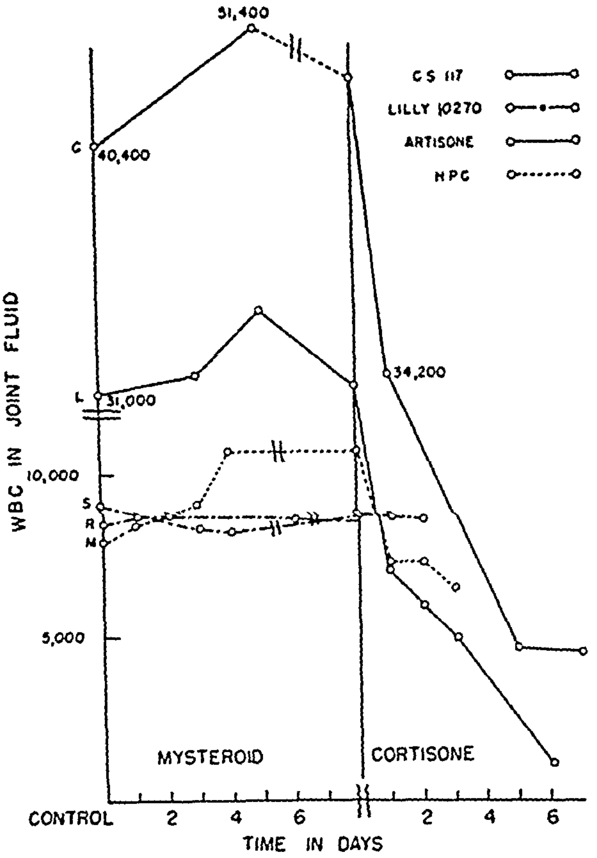

Fig. 6. The Comparative Effect of Administration of Other "Anti-arthritic" Agents and Cortisone on the Joint Fluid Cell Count in Four Cases of Rheumatoid Arthritis

Doses used were noted on Figure 5.

period of administration, much the same as previously noted from large doses of salicylates (6). Here again when cortisone was substituted the drop in joint temperature was more pronounced and objective clinical improvement was noted.

The correlated determination of cell count of the synovial fluid with this study is illustrated by Figure 6. Little change in the synovial fluid cell count was noted until cortisone was substituted for the "mysteroid," after which the cell count decreased in three out of four instances.

## DISCUSSION

With the advent of cortisone and ACTH, it became possible to bring about rapid clinical improvement in rheumatoid arthritis. Using these agents, and correlating clinical improvement with joint temperature measurement, a distinct parallelism was demonstrated. The experiments were limited to relatively short periods of observation, but appeared to demonstrate the prompt effectiveness of the agents under study. Whereas clinical determination of effect is difficult to evaluate on any but a large series of patients over considerable time, serial joint temperature determination might provide a rapid, relatively simple criterion for determining drug effectiveness in active rheumatoid arthritis.

The changes in joint temperature produced by administration and withdrawal of ACTH and cortisone seem greater than one would expect from circulatory variation alone. Changes in tissue metabolism in the synovium might account for much of the increased or decreased heat. This is still under study.

Moderate variations of room temperature have only a relatively minor effect on joint temperature (4). The electronic potentiometer recorded room temperature as well as simultaneous skin and joint temperatures. It will be noted from Figure 3 that an occasional break in the steady downward slope of joint temperature was recorded. In three of five such instances a restless night preceded the test, but as yet no definite explanation for the temporary elevation has been found. Likewise, the rise in joint temperature seen before stopping the hormones in seven cases may have indicated that the dose was inadequate, but this is not certain.

At least by the criterion of joint temperature measurement no other drug has been found so far which has any therapeutic effect at all comparable to ACTH or cortisone. By this method also, the effectiveness of orally administered cortisone has been found comparable with the parenteral preparation.

Because of the already described technical difficulties experienced in performing the joint fluid cell counts, the method for determining clinical effect objectively by drop in cells in the synovial fluid was found to be inferior to the more consistent determination of joint temperature.

## SUMMARY

Serial intra-articular temperature determinations were made on 21 rheumatoid arthritic patients before, during, and after a course of parenterally administered ACTH or cortisone. Cell counts were made on the synovial fluid obtained at the time of the aspirations for temperature determination in nine of the 21 patients. In every instance there was a pronounced fall in joint temperature within the first 24 hours of ACTH or cortisone administration, which approached normal by the fourth day of the treatment period. Joint fluid cell counts also showed a pronounced drop. Cortisone was administered orally to six patients with rheumatoid arthritis, with serial joint temperature determinations showing a similar fall in every instance. Eight patients with rheumatoid arthritis were given various other steroids for a period of one week, and serial joint temperature determinations made. Without the patients' knowledge cortisone was then substituted. None of these supposed anti-arthritic agents produced the fall in joint temperature which occurred in every case after the substitution of cortisone.

Serial joint temperature determination is suggested as a rapid and possibly specific laboratory method for establishing additional evidence of therapeutic effectiveness of anti-arthritic agents.

## ACKNOWLEDGMENTS

Acknowledgement is made to Dr. Francis C. Wood, Professor of Medicine, and to Dr. Francis D. W. Lukens, Associate Professor of Medicine, University of Pennsylvania, for their assistance.

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# THE EFFECT OF EPINEPHRINE ON THE PULMONARY CIRCULATION IN MAN[1]

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(Submitted for publication October 4, 1950; accepted April 16, 1951)

Extensive studies are available concerning the effect of epinephrine upon the lesser circulation of several species of animals and a variety of animal preparations. Until recently, this type of investigation has not been possible in man. The development of methods has now made such studies feasible, and much can be learned in a quantitative and semi-quantitative way. The intracardiac catheter, the dye dilution techniques for blood flow and volume, and a high frequency recording manometer are the corner-stones of the present study.

The purpose of this work was to discover what changes in pulmonary circulatory dynamics resulted from an intramuscular injection of epinephrine into a human. This aim was primarily clinical; therefore, dose, route of administration, and drug preparation were those commonly employed in therapy.

## MATERIALS AND METHODS

The patients were chosen at random from the medical and surgical chest services at Lawson VA Hospital. In age they ranged from 20 to 55 years. The average age was 31 years. All were male. Diagnoses and a summary of data are listed in Table I. None had clinical evidence of cardiovascular disease. With the exception of Cases 1, 2, 12, and 13, the pulmonary lesions were so sharply localized or limited in degree that no significant disturbance in the pulmonary circulation was suspected. The experiments dealing with intravenous and intramuscular injection of epinephrine were performed on these 13 patients in the post absorptive state.

The epinephrine[2] was a U.S.P. preparation and is said by its manufacturer to be a synthetic material, to exist 100 per cent in the levo-rotatory form as a hydrochloride, and to contain no traces of nor-epinephrine.

Pulmonary artery catheterization was performed in the usual way with a 6-F or 8-F intracardiac catheter. All systemic arterial blood samples and pressure records were obtained from an indwelling, femoral arterial needle. An electromanometer (Sanborn), recording through a single channel, direct-writing electrocardiograph (Sanborn), was used for all pressure tracings. Arbitrary reference point was 5 cm. below the sternal *angle of Louis.* Mean pressures were obtained by electrical integration of the pulse contours. Pulmonary "capillary" pressures were obtained as described by Hellems, Haynes, and Dexter (1).

Cardiac outputs were derived by the dye dilution method of Hamilton (2, 3), using Evans Blue dye (T-1824), and the technique of Dow and Pickering for determination of dye concentration (4). Dye output determinations done in this way have been shown to correlate well with the Fick procedure (5). The lesser, mean, and total circulation times, as derived from the graphic representation of the dye concentration curve, may be defined as follows: *Lesser (fastest) circulation time* is the appearance time (extrapolated) of the first detectable dye arriving at the femoral artery by the most rapid pathway. *Mean circulation time* is the mean of the appearance times of dye particles arriving at the femoral artery from all pathways during the dye's first complete circulation, and *total circulation time* is the time required for the dye column to completely circulate one time after having first reached the femoral artery (time of recirculation minus the lesser circulation time). A figure ("Q") was also derived from the dye curve as an index of pulmonary blood volume, as described by Ebert and associates (6). "Q" not only represents blood in the pulmonary vascular bed but also that in the left side of the heart, the aorta, and certain large arteries.

Although both Ebert and we ourselves have noted considerable scatter of values for "Q" in a series, we have also noted a surprising reproducibility in the same patient (7), even when the patient was in an unstable state. Figure 1 indicates that in the present study unless the "Q" value decreased by a very large amount, which we have attributed to the epinephrine, the majority of the figures changed less than 10 per cent in spite of gross changes in both cardiac output and mean circulation. In no case in which we have checked the procedure without disturbing the circulation, has there been a discrepancy of over 15 per cent, and almost all were within 10 per cent.

Vascular resistance and cardiac work were calculated according to the formulae mentioned by Dexter and associates (8).

The basic experiments were done as follows: The fast-

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[1] Reviewed in the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions published by the authors are the result of their own study and do not necessarily reflect the opinion or the policy of the Veterans Administration.

[2] Gold Leaf Pharmacal Company, New Rochelle, New York.

TABLE I

*Pulmonary circulation data before and after epinephrine*

| Case | Body surface area, *sq. m.* | Minutes after epinephrine | Cardiac output, *L/min.* | Cardiac index, *L/sq. m./min.* | Pulse, *rate/min.* | Stroke volume, *cc.* | Circulation time, *in sec.* | | | "Q", *in cc.* (pulm. blood vol. index) | Pulmonary artery pressure, *mm. Hg* | | | Mean pulm. "cap." press., *mm. Hg* | PA-"PC" gradient, *mm. Hg* | Pulmonary vascular resistance, *dynes sec. cm.$^{-5}$* | "Work" against pressure, *Kg. m./sq. m./min.* | | Diagnosis |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Lesser | Mean | Total | | Systolic | Diastolic | Mean | | | | Rt. ventricle | L. ventricle | |
| 1 | 1.62 | | 3.8 | 2.3 | 100 | 38 | 2.5 | 7.5 | 9 | 470 | 26 | 10 | 19 | 7 | 12 | 266 | .6 | 2.8 | Extensive bronchiectasis |
| | | 7 | 8.1 | 5.0 | 112 | 72 | 1.5 | 3.8 | 4 | 511 | 34 | 12 | 24 | 7 | 17 | 169 | 1.7 | 5.9 | |
| 2 | 1.91 | | 7.3 | 3.8 | 68 | 107 | 5.5 | 11.0 | 12 | 1333 | 33 | 8 | 18 | 4 | 14 | 154 | .9 | 4.6 | Tuberculosis, rt. pneumothorax |
| | | 10.5 | 10.3 | 5.4 | 75 | 138 | 3.5 | 8.4 | 10 | 1447 | 34 | 11 | 21 | 5 | 16 | 124 | 1.6 | 5.7 | |
| 3 | 2.06 | | 7.4 | 3.6 | 76 | 97 | 5.5 | 10.5 | 10 | 1288 | 12 | 1 | 7 | — | — | — | .4 | 4.0 | Normal |
| | | 11 | 10.3 | 5.1 | 100 | 105 | 3.5 | 8.0 | 8 | 1277 | 17 | 4 | 9 | — | — | — | .7 | 5.7 | |
| 4 | 1.58 | | 3.9 | 2.5 | 90 | 45 | 4.0 | 10.5 | 12 | 685 | 17 | 6 | 9 | 5 | 4 | 81 | .2 | 2.1 | Bronchiectasis |
| | | 8 | 5.8 | 3.7 | 96 | 61 | 3.3 | 7.5 | 10 | 725 | 19 | 6 | 12 | 5 | 7 | 96 | .6 | 4.2 | |
| 5 | 1.72 | | 4.8 | 2.8 | 80 | 60 | 4.5 | 12.3 | 15 | 980 | 15 | 4 | 8 | 3 | 5 | 83 | .3 | 3.1 | Tuberculosis, rt. upper lobe |
| | | 6 | 7.6 | 4.4 | 96 | 79 | 1.5 | 7.2 | 12 | 910 | 19 | 4 | 13 | 3 | 10 | 105 | .8 | — | |
| | | 19.5 | 6.6 | 3.8 | 90 | 73 | 2.0 | 7.5 | 11 | 830 | 16 | 4 | 11 | 3 | 8 | 91 | .5 | 3.6 | |
| 6 | 1.86 | | 5.2 | 2.8 | 70 | 52 | 5.5 | 16.6 | 16 | 1430 | 23 | 10 | 14 | — | — | — | .6 | 3.6 | Bronchiectasis, rt. lower lobe |
| | | 3 | 5.9 | 3.1 | 100 | 85 | 7.5 | 13.5 | 12 | 1340 | 27 | 12 | 21 | — | — | — | .9 | 4.8 | |
| 7 | 1.89 | | 4.8 | 2.6 | 60 | 81 | 3.5 | 11.0 | 14 | 807 | 15 | 7 | 11 | — | — | — | .4 | 2.9 | Tuberculosis, rt. upper lobe |
| | | 6 | 8.1 | 4.3 | 80 | 102 | 3.5 | 7.7 | 8 | 1040 | 23 | 7 | 14 | — | — | — | .9 | 4.9 | |
| 8 | 2.16 | | 5.5 | 2.6 | 84 | 66 | 5.5 | 12.8 | 14 | 1180 | 14 | 3 | 9 | — | — | — | .3 | 3.1 | Bronchiectasis |
| | | 3 | 8.4 | 3.9 | 100 | 84 | 4.5 | 9.2 | 11 | 1280 | 17 | 5 | 11 | — | — | — | .6 | 5.1 | |
| 9 | 1.62 | | 5.8 | 3.6 | 108 | 51 | 7.5 | 12.4 | 11 | 1200 | 10 | 5 | 7 | 2 | 5 | 73 | .4 | 3.7 | Emphysema, bronchiectasis, and asthma |
| | | 8.5 | 7.8 | 4.8 | 120 | 65 | 1.5 | 5.9 | 10 | 770 | 17 | 5 | 11 | 2 | 9 | 92 | .8 | 5.5 | |
| 10 | 1.70 | | 4.7 | 2.7 | 80 | 52 | 3.5 | 9.3 | 14 | 825 | 17 | 5 | 11 | — | — | — | .4 | 3.1 | Normal |
| | | 5 | 4.5 | 2.6 | 110 | 41 | 1.5 | 6.0 | 10 | 447 | 25 | 7 | 15 | — | — | — | .6 | 2.8 | |
| 11 | 2.06 | | 7.2 | 3.5 | 70 | 103 | 8.0 | 14.5 | 14.4 | 1730 | 19 | 5 | 10 | — | — | — | .5 | 5.5 | Normal |
| | | 4 | 7.8 | 3.8 | 90 | 86 | 2.0 | 8.7 | 11.5 | 1130 | 25 | 7 | 17 | — | — | — | .9 | 5.7 | |
| 12 | 1.78 | | 7.7 | 4.3 | 80 | 97 | 5.5 | 10.5 | 12 | 1350 | 16 | 6 | 12 | — | — | — | .7 | 5.7 | Asthma, emphysema |
| | | 7 | 6.4 | 3.6 | 85 | 76 | 2.5 | 8.0 | 8.2 | 877 | 30 | 12 | 20 | — | — | — | 1.1 | 4.0 | |
| 13 | 1.53 | | 3.8 | 2.6 | 96 | 40 | 5.5 | 13.5 | 14 | 858 | 21 | 6 | 13 | — | — | — | .5 | 3.1 | Chronic lung abscess—pulmonary fibrosis |
| | | 2.5 | 6.5 | 3.9 | 120 | 54 | 1.5 | 6.5 | 8 | 722 | 30 | 12 | 17 | — | — | — | .9 | 6.2 | |

ing patients were sedated with .1 or .2 gm. of sodium phenobarbital intramuscularly, the catheter placed in the pulmonary artery, and the indwelling, femoral arterial needle inserted. A preliminary dye injection was made and several pressure tracings taken. Then 0.5 to 0.7 cc. of 1:1000 dilution of epinephrine was injected intramuscularly, and pressures were closely followed. From two and one-half to 10 minutes later, a second dye injection was made. In most instances, the pressures were followed until their return to normal. In one case, a third dye injection was done. In addition, 0.025 mg. of epinephrine was given intravenously on six occasions, and continuous tracings of either the femoral, pulmonary arterial, or pulmonary "capillary" pressures were recorded.

## RESULTS

### *Pressure Changes in the Pulmonary Circulation*

Under basal conditions at the beginning of the studies, the average pulmonary artery pressure for our 13 patients was 18.6 with a mean value of 11 mm. Hg. Mean "capillary" pressures in five patients averaged 4 mm. Hg with a range of from 2 to 7 mm. Hg. These pressures are generally lower than those found in some normal series (8). The reason for this difference probably lies in the arbitrarily chosen reference points. Our average pulmonary artery-pulmonary "capillary" gradient was 8.6 mm. Hg with a range of from 4 to 14 mm. Hg.

The mean pulmonary artery pressure rose in every case following the injection of epinephrine. The average rise of the mean pulmonary artery pressure was 4.4 ± .54[3] mm. Hg with a range of 2 to 8 mm. Hg. This rise was due chiefly to an elevation of systolic pressure which rose, on the average, 6 mm. Hg with a range of 2 to 14 mm. Hg. The average diastolic rise was only 2 mm. Hg with a range of 0 to 6 mm. Hg. The only two patients who had a striking diastolic rise (6 mm. Hg each) were among those four who were judged to have severe chronic lung disease but no pulmonary hypertension. The other two such patients had mild pulmonary hypertension but had diastolic rises of only 2 and 3 mm. Hg each.

The pulmonary pressure rise usually began in one to two minutes after the intramuscular injection, reached a peak in two to 16 minutes (average 7 mm. Hg), and gradually returned to basal levels in about 20 minutes. In three patients, however, the return to normal was not so prompt, and pressure was still elevated at 30, 32 and 46 minutes after injection. In these patients, no clinical or subjective signs of epinephrine effects were then present, with the exception of a slightly elevated pulse rate.

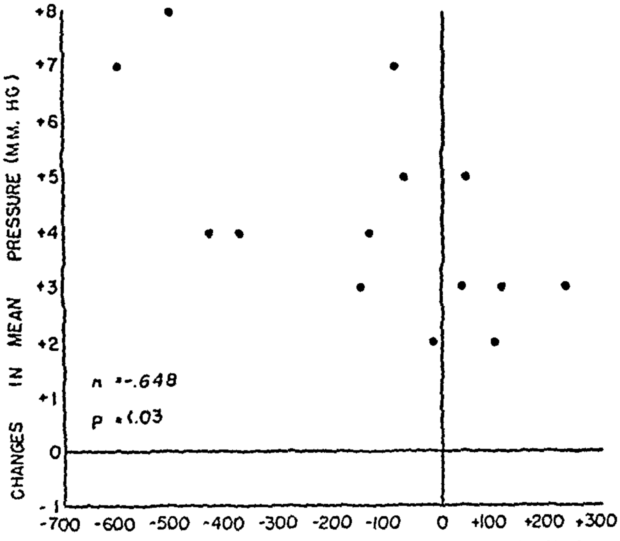

FIG. 1. RELATION BETWEEN CHANGES IN THE MEAN VALUES OF PULMONARY BLOOD VOLUME INDEX AND PULMONARY ARTERY PRESSURES AFTER ADMINISTRATION OF EPINEPHRINE

When the index of pulmonary blood volume ("Q") was determined at a time when the epinephrine-induced pulmonary hypertension was at its peak (in five cases), the pulmonary blood volume index had fallen considerably below its initial level. Also, in these five cases, the changes in cardiac output were much less than in the remaining eight cases.

The pulmonary "capillary" pressure measured in five cases rose very slightly if at all. The average pressure at the time of the second set of determinations was only 0.5 mm. Hg higher than the average before epinephrine. This average figure, derived from single readings, sometimes temporally distant from the peak effect, however, obscures the fact that there was a 2 to 3 mm. Hg rise in pulmonary "capillary" pressure in most cases coincident with the peak rise in mean pulmonary artery pressure. It was never of sufficient degree to account for the total elevation in mean pulmonary artery pressure and, as in Case 2 (Figure 2), had disappeared, while the pulmonary artery pressure was still high.

The pulmonary artery-pulmonary "capillary" gradient, however, had increased quite sharply in four cases and slightly in the fifth at the times at which it was measured. The average increase was 3 mm. Hg higher after epinephrine.

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[3] All values are expressed as mean ± standard error of the mean.

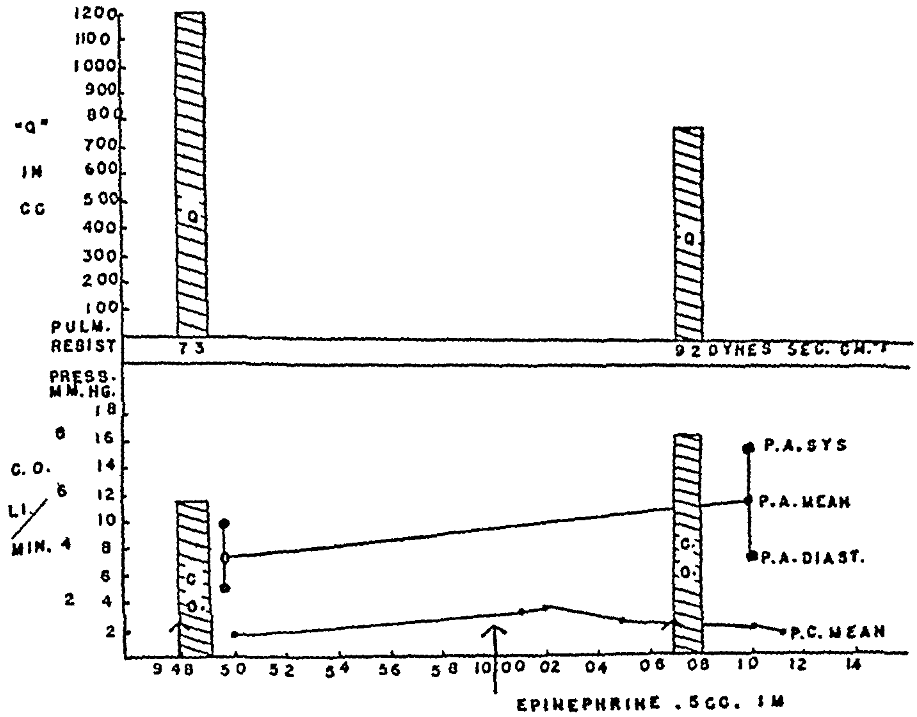

FIG. 2. HEMODYNAMIC CHANGES FOLLOWING EPINEPHRINE INJECTION AS OBSERVED IN CASE 9

C.O. = cardiac output
"Q" = index of pulmonary blood volume
P.A. Sys. = pulmonary artery systolic pressure
P.A. Mean = pulmonary artery mean pressure
P.A. Diast. = pulmonary artery diastolic pressure
P.C. Mean = pulmonary "capillary" mean pressure

Calculated figures for pulmonary vascular resistance are written beneath "Q" block graphs. Time of injection of epinephrine is represented by large arrow at time denoted as 10:00 o'clock. Note that "Q" decreased considerably in the presence of elevated pulmonary resistance, pulmonary artery pressure, and cardiac output.

An attempt was made to determine whether a direct effect of epinephrine on the pulmonary artery could be demonstrated by injecting 0.025 mg. of epinephrine directly into the antecubital vein and noting the time required for the expected rise in pulmonary artery pressure to occur. The procedure was then repeated in the same patient while a continuous tracing was made of the pulmonary artery, capillary, or femoral artery pressure. If the intravenously injected epinephrine had had a direct effect on the pulmonary vascular bed in its first passage through it, a rise in pulmonary artery pressure should be expected in eight to 10 seconds (9). Only one of three patients showed a detectable response at a time (11 seconds) approaching this, and that patient also had just as early a response in femoral artery pressure. Therefore, we were unable to demonstrate by this rough method and with this dosage, a direct effect of epinephrine on the pulmonary vascular tree. Doyle, Wilson, and Warren (10), using a similar technique, have shown that in four of a group of five patients there was a rise in pulmonary artery pressure within 10 to 12 seconds after the intravenous injection of epinephrine. Other studies with simultaneous ballistocardiograph and femoral and pulmonary artery pressure tracings, after a small intravenous dose of epinephrine, should be done.

### *Cardiac Output, Pulse Rate, Stroke Volume*

Before epinephrine, our initial cardiac indices averaged 3.0 L/min./sq.m. with a range from 2.3 to 4.3, which compares favorably with basal patients studied by the Fick method (11). Two and one-half to 11 minutes after the intramuscular injection of epinephrine the mean rise of the cardiac index was 1.0 ± 0.1 L/min./sq.m. There was a rise in 11 of the 13 patients, no change in one (Case 10), and a definite fall in another (Case 12). There was no apparent reason for the failure of the index to rise in Case 10. Case 12, however, was rather anxious and had a high initial cardiac output which probably masked any change that might have been caused by the epinephrine. There was, in addition, very little acceleration of the pulse in this patient after epinephrine in spite of a definite change in circulation time.

Pulse rate increased from five to 30 beats per minute following epinephrine. Averages before and after epinephrine were 82 and 98 respectively. A rise was noted in all 13 cases.

Stroke volume increased in 10 of 13 cases. Increases ranged from 8 to 90 per cent with an average rise of 26 per cent. In the other three cases, there was a moderate fall of from 16 to 22 per cent.

### *Circulation Times*

Speed of circulation of the dye from pulmonary to femoral artery was increased in every instance following epinephrine. This was not so apparent in the "lesser" circulation time because sampling of femoral arterial blood was done only at two-second intervals, and the smaller absolute changes might have been partially missed. Average resting values for the lesser, mean, and total circulation times were 5, 11.7, and 12.8 seconds respectively. After epinephrine, these values dropped to 2.9, 8, and 9.4 seconds. There was an impressive acceleration of blood flow in every case when measured by the mean circulation time.

### *Pulmonary Blood Volume Index or "Q"*

The average "Q" for our 13 patients under basal conditions was 1087 cc. ranging from 470 to 1730 cc. These figures are slightly lower than the figure given by Ebert and colleagues (6). One explanation may be that Ebert's group was a more nearly normal group. Moreover, the cardiac outputs and mean circulation times used in the formula for "Q" by Ebert were not simultaneous, since the outputs were done by the Fick procedure and the circulation time derived from a dye curve obtained afterwards. There may have been changes in the circulation time or cardiac output between the two procedures.

The average "Q" after epinephrine was 959 cc., an average decrease of 128 ± 72 cc., which is not significant. It must be remembered that usually only one dye dilution curve, covering a period of about 20 seconds, was done on each patient after the epinephrine injection. Transient changes might or might not have coincided with the dye procedure. This, indeed, seems quite probable when individual protocols are studied. Figure 1 illustrates a significant correlation between the height of the pulmonary artery pressure rise and the change in "Q." If the procedure was done when the mean pulmonary artery pressure was quite high, "Q" was apt to have fallen; if the determination was done before or after the peak rise in pressure, the "Q" value remained nearly the same. Figure 1 reveals five cases (Cases 9–13) in which "Q" fell from 16 to 46 per cent (150 to 600 cc.), well outside the usual variation of 10 per cent.

If these five experiments in which there was a considerable fall in "Q" are separated and compared with the remaining eight cases in which "Q" showed little change, it will be found that there is no great difference between the two groups in regard to the change in pulse rate and circulation time; also, the temporal relation between the determinations and the epinephrine injection was the same. In addition to the relationship illustrated in Figure 1, there is a great difference between this group of five cases and the remaining eight patients in regard to change in cardiac index and stroke volume. Among these five cases in which "Q" fell, only one had a large increase in cardiac index; the average rise in cardiac index was only 8 per cent. Average stroke volume rise was equally insignificant (0.6 per cent). In the remaining eight cases, however, the average rise in cardiac index was over 50 per cent and in stroke volume, over 30 per cent. The relationship of the changes of the cardiac index to the changes of the pulmonary blood volume index is graphically expressed in Figure 3. A positive correlation of stroke volume with pulmonary blood volume has been noted previously under physiological conditions in man (3).

In summary, five of 13 patients had a fall in "Q" following epinephrine administration, which was concomitant with the peak rise in pulmonary artery pressure. Four of these five cases had negligible changes in cardiac output, whereas most of the

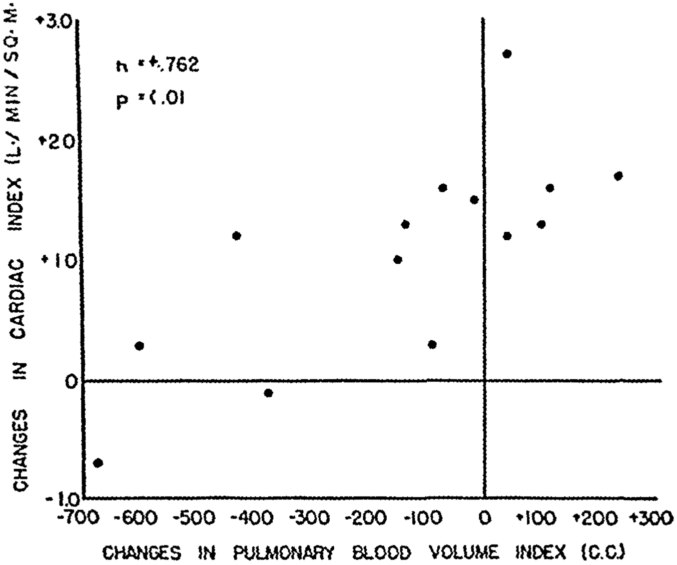

FIG. 3. RELATION BETWEEN CHANGES IN THE MEAN VALUES OF THE CARDIAC INDEX AND THE VOLUME OF BLOOD WITHIN THE LUNG ("Q"), FOLLOWING EPINEPHRINE ADMINISTRATION

When the output has changed little, the pulmonary blood volume index tends to fall; when the output increases, the index of pulmonary blood volume tends to remain the same or increase.

remaining eight cases had marked increases in cardiac output with relatively little change in "Q."

### *Pulmonary Vascular Resistance and Right Ventricular Work*

In five cases (Cases 1, 2, 4, 5, 9), the pressure gradient between pulmonary artery and pulmonary "capillary" was determined before and after epinephrine and pulmonary vascular resistance calculated. This figure rose 26, 19, and 27 per cent in three cases (Cases 4, 5, 9) and fell 40 and 26 per cent in the other two cases (Cases 1, 2).

Looking at these cases individually, we see that all of the first mentioned patients had relatively mild disease and had little change in diastolic pressure after the drug. Calculated basal vascular resistances were within Dexter's normal range (8). The other two patients had severe lung disease, had mild pulmonary hypertension at rest (the two highest of all the 13 patients), and definitely had a rise in diastolic pressure after epinephrine. One of the two also had the very low "Q" value of 470 cc. (the lowest in the entire series). The resulting values for pulmonary vascular resistance in these two were markedly elevated at rest when compared with the other three or with Dexter's normal values.

To summarize this information, the three patients with relatively normal pulmonary vascular beds showed evidence of an increase in pulmonary vascular resistance following epinephrine. In two patients with considerable evidence of chronic lung disease and probable pulmonary vascular disease, there was a considerable fall in pulmonary vascular resistance.

The work of the right ventricle against pressure increased sharply in every case, from 30 to 174 per cent, with an average of 99 per cent. This result was not surprising since in almost every instance there was a rise in both pulmonary artery pressure and in cardiac output.

### *Left Ventricular Work, Systemic Arterial Pressure, and Peripheral Resistance*

Since we were almost constantly recording pressure in the pulmonary circuit with our single manometer, it was impossible to study the pressure responses in the femoral artery adequately. A special point, however, was made of taking such a recording immediately following the dye injection so that peripheral resistance and left ventricular work could be calculated. Otherwise, our information was not adequate to draw conclusions about the systemic pressure response in these experiments. Such studies have been done (12), and our impression is that our cases would have shown nothing unexpected. In every case, the pulse contour suggested a decrease in peripheral resistance, and this was corroborated by calculation. There was a slight rise of 5 to 15 mm. Hg in the systolic pressure in a few cases, and the mean pressure rose slightly in six patients. Diastolic pressure fell slightly or did not change. Mean pressures fell slightly in six cases. No recording was made in one.

Left ventricular work rose in 10 of 12 cases where it could be calculated, but the average rise was only 25 per cent, about half the percentage rise of work in the right ventricle. In absolute terms, however, the changes in left ventricular work against pressure were considerably more than the changes occurring in the right ventricle.

## DISCUSSION

### *Effect of Epinephrine on Pulmonary Blood Volume*

Using dye injection methods, Hamilton has demonstrated that large doses (.1 to .5 mg.) of epinephrine, injected intravenously into the intact dog under morphine anesthesia, result in a slowing of the pulse rate and circulation time, a fall in cardiac output, and an increase in the quantity of blood encompassed by the figure "Q" (13). This has been explained as the result of the inability of the left ventricle to increase its output against a suddenly increased pressure; as a consequence, blood dams back into the pulmonary reservoir and in the right side of the heart and in the veins. Wearn and associates have, by direct observation in animals, noted the opening up of new capillaries and engorgement of small pulmonary blood vessels after similar doses of epinephrine (14). It seems reasonable to believe that with these large doses in the dog, an increase in the volume of blood within the lung does occur.

With small doses of epinephrine, however, the evidence is to the contrary. By direct observations, Hall found a rapid pulse and alveolar capillaries which became thinner and contained less blood (15).

In the heart-lung preparation very small doses invariably increased heart rate and blood flow and decreased the pulmonary blood volume (16). Daly concluded that there was fairly good evidence from several sources that epinephrine caused a decrease in size in the large extrapulmonary portions of the pulmonary vascular system (17).

In a number of cases, we have demonstrated that the circulating pulmonary blood volume index probably drops precipitously for a short time after a small intramuscular dose of epinephrine. Since only about 60 cc. of blood are contained in the human pulmonary capillaries (18) and, of course, a very small amount in the precapillaries, the drop in "Q" of several hundred cubic centimeters probably cannot be explained on the basis of changes in the volume of these sections of the pulmonary vascular bed. Changes of this magnitude must reflect a decrease in the volume of the large pulmonary arteries and veins regardless of what is happening in the "arterioles" or "capillaries." If an increase in pulmonary vascular resistance reflects a decrease in size of the pulmonary "arterioles," our observations on a few cases indicate the lack of relations between total pulmonary blood volume and size of the "arteriolar" bed in these cases. In Figures 2 and 4, two cases are shown in which the vascular resistance definitely increased. In one, however, there was apparently a considerable decrease in pulmonary blood volume index, but in the other there was no change.

The question arises whether this change in "Q"

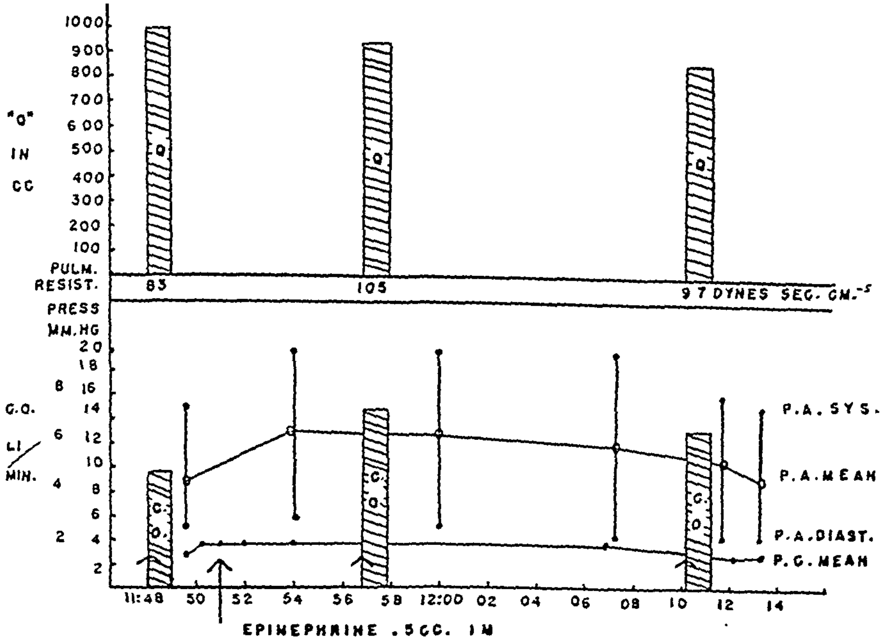

FIG. 4. HEMODYNAMIC CHANGES FOLLOWING EPINEPHRINE, AS OBSERVED IN CASE 5

Symbols are the same as in Figure 3. In this case, "Q" did not change appreciably, although all other values represented did increase.

might be due partially to changes in the extrapulmonary components that make up the total value. It is true that the size of the left heart might become smaller after epinephrine, but it seems unlikely that a normal heart would shrink enough to account for a drop of several hundred cubic centimeters. Little is known about the reaction of the living human aorta to epinephrine, but the evidence is that small doses probably dilate certain large arteries such as the femoral (19). This would actually tend to increase the "Q" value.

In his experiments with epinephrine, using dogs, Hamilton (13) found a prolonged dye dilution curve which indicated a low output and slow circulation. Using comparatively much smaller doses in the human, we have found dye curves of short duration which indicate increased outputs and rapid circulation. In Hamilton's experiments, heart rates were slowed, but in ours they were accelerated. Our epinephrine dye curves very closely resembled those in Hamilton's dogs following amyl nitrite. Apparently, circulatory dynamics in such experiments also closely approached those in ours. After amyl nitrite there was an increase in cardiac output, rapid circulation, accelerated heart rate, a fall in systemic peripheral resistance, and a decreased intrathoracic blood volume.

There is ample evidence from many sources that in therapeutic doses in man, epinephrine is a general vasodilator drug. This is apparent from pulse contours, fall in peripheral resistance, and direct effect on large arteries (12, 20–22). There is a profound redistribution of blood flow, the best known examples of which are the blanching of the skin and the increased flow to skeletal muscle (19).

It seems possible that the mechanisms whereby amyl nitrite and small amounts of epinephrine cause the heart to pump blood from the lesser into the greater circulation may be similar. In both cases the lung is giving up blood to widely dilated vascular areas. There is, nevertheless, one great difference in the action of the two drugs. Although their peripheral actions are similar, epinephrine has a stimulating effect on the myocardium that amyl nitrite lacks. McMichael and Sharpey-Schafer have shown that with very small doses this effect may be evident in an increased stroke volume without change in pulse rate or blood pressure (23). This implies a separation of the central and peripheral actions of the drug and suggests why in our experiments the fall in pulmonary blood volume index is probably so inconstant. When cardiac output increase is substantial, as in most of our cases, the dilated areas can be served without calling on the pulmonary circulation, but when the peripheral action of the drug is the more pronounced, there is relatively little change in output, and the pulmonary vascular circuit is drained to supply the dilated peripheral arterial beds. This explanation fits in with the fact that in our experiments demonstrating a fall in pulmonary blood volume index, there was very little or no increase in cardiac output and in stroke volume, although a fall in peripheral resistance and circulation time, and an increase in pulse rate had taken place. In the other cases, however, there was a decided increase in stroke volume and minute output, and the pulmonary blood volume index did not change.

### *Effect on the Pressures in the Pulmonary Circuit*

The most consistent effect of epinephrine on the lesser circulation has been a rise in pulmonary artery pressure. The pressure in this system is continually influenced by the output of the right ventricle, the effect of back pressure in the pulmonary veins, and peripheral resistance in the pulmonary circuit (9). To elevate the pulmonary artery pressure in the normal human by increasing the cardiac output is difficult. When this is done by exercise, a minimum cardiac index of 5.5 to 6.0 is necessary (8, 24). These values were not approached in our cases. In addition, the largest increases in pulmonary artery pressure, which were accompanied by a fall in "Q," had the smallest increases in cardiac output. Although an increase in blood flow might tend to increase the pressure, it is an insufficient reason to explain the rise in the present experiment.

Hamilton's experiments with dogs given large doses of epinephrine clearly indicated that the pulmonary artery pressure increases so recorded were simply a reflection of an increased pulmonary venous pressure and that no change in the pressure gradient between the pulmonary artery and vein occurred (25). This does not seem to be an adequate explanation in the present experiments, because in the cases in which it was measured, there was very little change in the pulmonary "capillary"

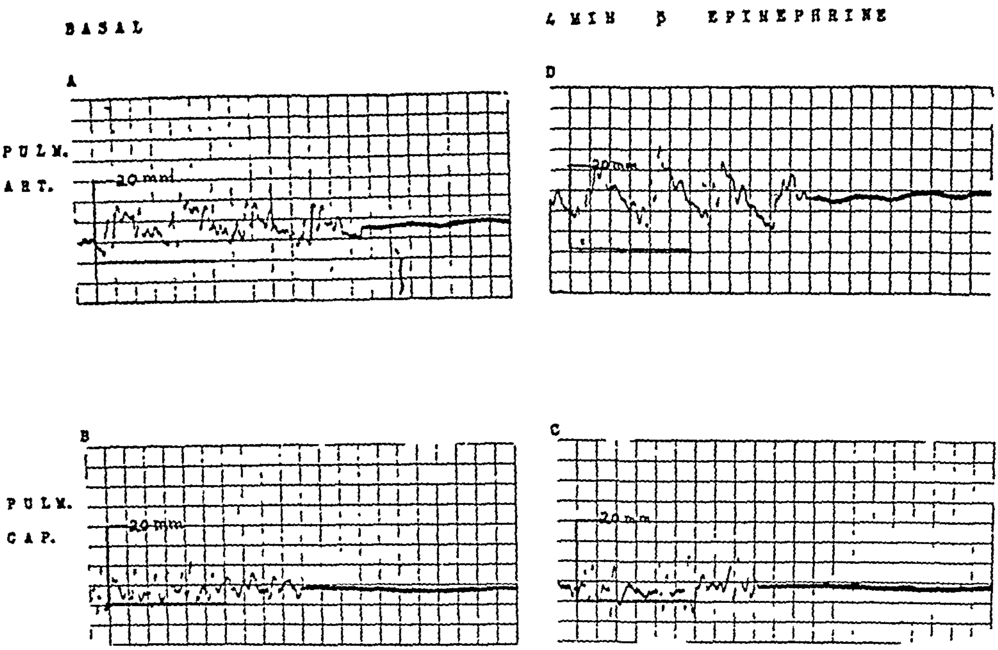

FIG. 5. SAMPLE TRACINGS FROM EXPERIMENT ON CASE 9 (Figure 3).

At the left are pulmonary artery and pulmonary "capillary" tracings taken shortly before the injection of epinephrine. Similar tracings 10 minutes after epinephrine are seen on the right. The dampened portions of the records are electrically integrated mean values. Standardizations and base lines have been copied into the left corner of each record. Time interval between C and D was less than 15 seconds.

pressure, and consequently an increased gradient resulted. In the cases in which the pulmonary "capillary" pressure was not measured, there was relatively little change in the diastolic pressures after epinephrine, and since a relation between pulmonary artery diastolic and pulmonary "capillary" pressure has been established (8), the pulmonary "capillary" pressure probably did not rise very much in this group either. The exceptions to the previous statement are the patients with widespread chronic lung disease.

Hamilton has pointed out that changes in the pressure gradient across the pulmonary circuit are considerably more important than the actual level of pressure in the pulmonary artery in determining whether peripheral resistance has changed or not (26). A sudden large increase in the cardiac output might conceivably widen the gradient, but cardiac output changes of the order of magnitude that we have found do not. The only explanation remaining, that there has been a decrease in the cross sectional areas of the terminal "arterioles," is likely (27). Hall's observations suggest the same conclusion (15). In three relatively normal cases (Figures 3 and 4), a moderate but definite increase in pulmonary vascular resistance has been demonstrated. Pressure records of one of these are seen in Figure 5. In the two cases with chronic lung disease and resting pulmonary hypertension, an entirely different result occurred. The elevated figures for pulmonary vascular resistance at rest fell considerably after epinephrine. This would be more difficult to understand were we to believe that the high pulmonary artery pressures and resistance were due to irreversible organic narrowing of the terminal "arterioles" and precapillaries. Cournand and his coworkers have recently shown quite definitely that the pulmonary hypertension of chronic lung disease is reversible to a large extent (28). This suggests that the high resistances in these cases might well have been due in part to vasoconstriction. In addition, Hickam (29) and Dexter and associates (8) have given evidence that the pulmonary hypertension of left ventricular failure may be partially due to an increased peripheral resistance within the circuit. The different response to epinephrine in normals and in two patients with mild pulmonary hypertension suggests the possibility that the action of epinephrine upon the small arborizations of the pulmonary artery is dependent upon their state of contraction at the time the drug is given.

Finally, what is the mechanism by which epinephrine acts upon the small end branches of the pulmonary artery? We have failed to produce convincing evidence that it acts directly upon the blood vessels of the lung while on its first circulation in fairly high concentration. Our intravenous experiments are admittedly crude, however, and dosage small. The evidence for reflex vasomotor control of the human pulmonary circulation is "by and large" non-convincing (9), and we can add nothing to this argument. With the methods now available, such evidence should be forthcoming.

## SUMMARY

1. Epinephrine, uncontaminated by nor-epinephrine, in intramuscular doses of from 0.5 to 0.7 cc. of a 1 : 1000 solution causes the following events to occur in the pulmonary circulation:

a. A decrease in the intrapulmonary circulation time.

b. A decrease in pulmonary blood volume index under certain circumstances when its action is suggestively that of a peripheral vasodilator and central effect is minimal.

c. A rise in the systolic and mean pulmonary artery pressures. The diastolic pressures are usually increased very little. Pulmonary "capillary" pressures are usually slightly elevated also, but the degree and duration is not sufficient to account for the rise in pulmonary artery pressure. The moderate increases in cardiac output are also insufficient to account for the rise.

2. In three cases, it was evident that an increase in pulmonary vascular resistance was the primary cause for this epinephrine-induced pulmonary hypertension.

3. In two patients with severe chronic lung disease, a greater increase in diastolic pressure occurred, and pulmonary vascular resistance was found to fall after epinephrine.

4. By the method used, it could not be demonstrated that the effects were due to a direct action of the drug on the pulmonary vascular tree, and the mechanism of this phenomenon remains obscure.

5. The work of the right ventricle against pressure increases proportionately much more than that of the left ventricle.

## ACKNOWLEDGMENTS

The authors wish to express their appreciation to Mrs. Barbara Wall, Mrs. Juanita Lewis, and Miss Beth Park for technical assistance, to Dr. William F. Hamilton, Dr. James V. Warren, and Dr. Walter H. Cargill for their suggestions and criticisms, and to Dr. Philip K. Bondy for his assistance in the statistical analyses.

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# ELECTROPHORETIC STUDIES IN LIVER DISEASE. I. COMPARISON OF SERUM AND PLASMA ELECTROPHORETIC PATTERNS IN LIVER DISEASE, WITH SPECIAL REFERENCE TO FIBRINOGEN AND GAMMA GLOBULIN PATTERNS[1, 2]

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(Submitted for publication January 5, 1951; accepted April 23, 1951)

The development of electrophoresis has permitted the study of body-fluid protein changes in health and disease. Most reports on electrophoretic analysis in hepatic diseases are based upon findings in serum rather than plasma. The chief reasons for the preference of serum over plasma are: (*a*) it avoids the use of anticoagulants such as heparin which may affect the electrophoretic pattern (1), (*b*) it permits comparison of electrophoretic data with the usual flocculation tests performed on serum, (*c*) it avoids the difficulty of differentiating protein complexes having a mobility similar to that of fibrinogen and forming with it a single peak which is incapable of resolution into its constituent components (2).

In a recent study of electrophoretic plasma protein partition of patients with hepatic diseases, especially those with cirrhosis, unexpected fibrinogen and gamma globulin values were noted. The "fibrinogen" peaks were markedly elevated and the gamma globulin elevations were not as high as expected from previous serum electrophoresis and chemical partition. To clear up these discrepancies, electrophoretic tracings on a series of simultaneously collected serum and plasma specimens of normal persons and patients with various hepatobiliary and other diseases were compared with each other as well as with chemical partition of blood proteins. This study was planned to give general information on possible differences in the electrophoretic pattern of serum and plasma of normal persons as well as patients with hepatic and other diseases.

## MATERIALS AND METHODS

### *Clinical Material*

The subjects for the study came from three groups: (*1*) a group with liver disease made up of 23 cirrhotics (22 of the Laennec type and one post-necrotic), six cases of infectious and six of toxic hepatitis (two arsenic, one post-pneumonic and three of unknown etiology whose clinical and biopsy pictures resembled toxic hepatitis of known toxic etiology) and three cases of extrahepatic obstruction (two pancreatic neoplasms and one case of common duct lithiasis); (*2*) a miscellaneous group of 15 cases of rheumatoid arthritis, two of gastric ulcer and one of multiple myeloma; and (*3*) a normal group composed of 10 doctors and technicians working in the laboratory.

Hepatic tests were performed upon all subjects. In addition all but five of the patients with hepatic disease had needle or surgical biopsies performed for confirmation of the diagnoses. All diagnoses were made by clinical, laboratory or surgical means.

### *Method*

Approximately 50 ml. of blood were drawn from every subject in the fasting state. Ten ml. were placed in a dry clean test tube and allowed to clot, after which the serum was separated by centrifugation. Another 10 ml. were placed in a test tube containing 30 mg. of potassium oxalate as the anticoagulant and centrifuged for separation of plasma. The remainder was utilized for chemical determination of the total serum proteins, fibrinogen (in 27 cases), or protein partition, and other hepatic function tests.

Electrophoretic analysis of the pairs of serum and plasma was performed in a Tiselius apparatus at pH 8.6 sodium diethyl barbiturate and ionic strength of 0.1

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[1] A preliminary report of this investigation was included in the Proceedings of the Twenty-Second Annual Meeting of the Central Society for Clinical Research (Proc. Cent. Soc. Clin. Res., 1949, 22, 28).

[2] This investigation was supported in part by grants from the Commission on Liver Disease, Army Epidemiological Board, Office of the Surgeon General, U. S. Army, Washington, D. C., and from the Dr. Jerome D. Solomon Memorial Research Foundation, Chicago, Illinois.

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with barbiturate buffer according to the Longsworth procedure (3). All runs were made at 1.5° C. for 120 minutes. Schlieren diagrams, photographically enlarged, were traced with a planimeter according to the method of Pedersen and Svedberg (4), to obtain the percentage of the total area assigned to each protein fraction. Mobility measurements were made from the boundary anomaly of the pattern. Only descending patterns were used. The relative concentrations, calculated from the proportions of the component peaks of the patterns, were converted to absolute concentrations expressed in grams/100 ml. of the total serum protein which had been determined chemically. Total serum proteins were determined by the Weichselbaum modification of the biuret reaction (5). The nitrogen to protein conversion factor used was 6.25. In 27 instances fibrinogen was determined chemically by standard laboratory methods of calcium salt precipitation and nitrogen determination (6).

Total plasma proteins were not determined chemically. The normal values for serum are practically identical with those of plasma despite a fibrinogen content of 200–400 mg./100 ml. in the latter (7, 8). The addition of an anticoagulant increases the plasma salt concentration and causes diffusion of water out of the erythrocytes. The presence of fibrinogen in plasma is thus nullified by the increased dilution of the plasma (9). When comparing absolute plasma and serum findings it is desirable to reduce this source of error. A formula was utilized to calculate the total plasma proteins from the absolute serum protein values and the relative percentages of the serum and plasma electrophoretic components. The formula and its derivation are as follows:

$N$ = total plasma protein in grams/100 ml.
$S$ = total serum protein in grams/100 ml.
$X$ = plasma fibrinogen content in grams/100 ml.
$S = N - X$
$\gamma'$ = grams of $\gamma'$ component found in serum

$$g = \frac{\%\ \gamma' \text{ component in serum (electrophoretic)}}{100} = \text{fraction of } \gamma' \text{ found in serum}$$

$$\gamma' = gS$$

$$f = \frac{\%\ \phi \text{ (electrophoretic)}}{100} = \text{fraction of } \phi \text{ found in plasma.}$$

$$X = fN - \gamma'$$

$$S = N - (fN - \gamma').$$

$$N = \frac{S - \gamma'}{1 - f}$$

## RESULTS

The detailed analyses of our electrophoretic findings in the various liver diseases and their correlation with the clinical, chemical and biopsy findings are being reported elsewhere. This report is concerned primarily with the difference between serum and plasma in different liver diseases. These are apparent in Figure 1 which depicts the

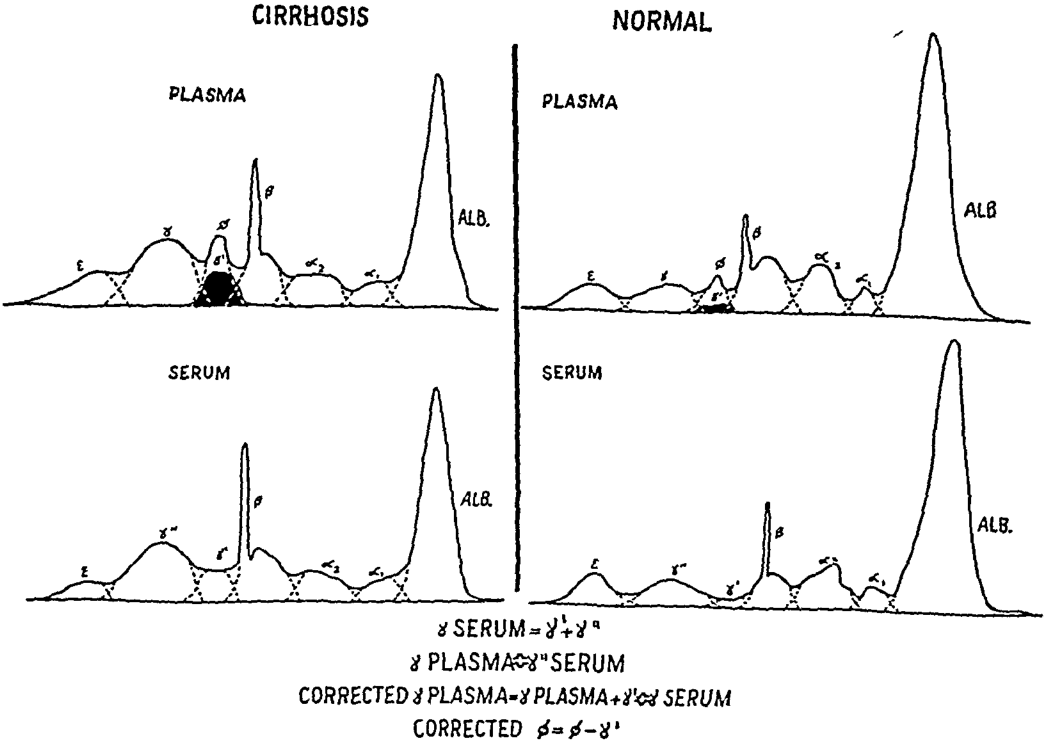


FIG. 1. ELECTROPHORETIC PATTERNS ON PLASMA AND SERUM OF A CIRRHOTIC AND NORMAL INDIVIDUAL

typical serum and plasma electrophoretic patterns in normal and cirrhotic subjects. The usual findings of decreased albumin and increased gamma globulin (10–15) are seen in the cirrhotic pattern and the only differences between the serum and plasma patterns are in the gamma globulin and fibrinogen fractions. The normal patterns show similar serum-plasma differences except they are smaller. These serum-plasma differences in the globulin and fibrinogen components will be described and discussed later. The descending patterns are used. In addition to the presence of the usual peaks denoting the albumin, alpha$_1$, alpha$_2$, beta, gamma, fibrinogen, and epsilon boundary anomaly, the gamma globulin component is composed of a fraction labelled gamma$_2$, and a gamma$_1$ fraction of greater mobility. The gamma$_1$ portion of the plasma gamma globulin is buried in the "fibrinogen" peak so that the plasma gamma globulin peak corresponds to the gamma$_2$ component of the serum. We have adopted the nomenclature of Deutsch (2) in describing the globulin components.

The mean percentage distributions, absolute concentrations and mobilities of the protein components as determined electrophoretically in serum and plasma are summarized in Tables I and II.

TABLE I

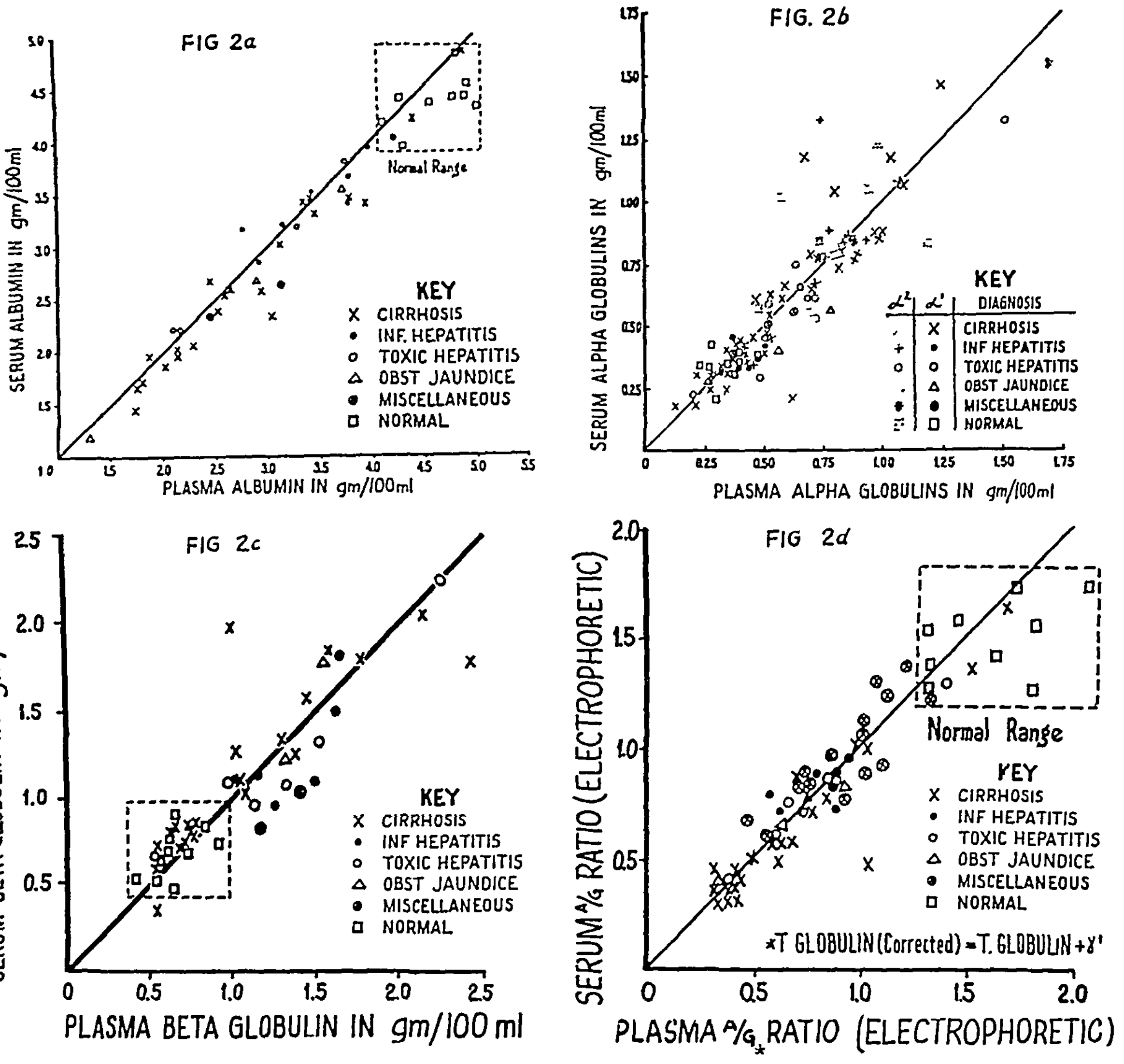
*Electrophoretic analyses of serum and plasma in normal individuals and in patients with liver and miscellaneous diseases*

| DIAGNOSIS | No. of Cases | Serum Plasma | $TP_S$ | $TP_P$ calc. | Albumin | | Globulin | | Globulin corr. | | $\alpha_1$ | | $\alpha_2$ | | $\alpha$ TOTAL | | $\beta$ | | $\gamma'$ | | $\gamma''$ | | $\gamma_s$ TOTAL | | $\gamma_p$ | | $\gamma_p$ corr. | | $\phi$ | | $\phi$ corr. | | A/G | A/G$_P$ corr. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | gm/100 ml | | gm/100ml | %T.P | gm/100ml | %T.P | gm/100ml | %T.P | gm/100ml | %T.P | gm/100ml | %T.P | gm/100ml | %T.P | gm/100ml | %T.P | gm/100ml | %T.P | gm/100ml | %T.P | gm/100ml | %T.P | gm/100ml | %T.P | gm/100ml | %T.P | gm/100ml | %T.P | gm/100ml | %T.P | | |
| CIRRHOSIS | 23 | S | 706 | | 2.47 | 35.0 | 4.59 | 65 0 | | | 0.40 | 5.7 | 0.80 | 11.3 | 1.20 | 17.0 | 1.22 | 17.3 | 0.50 | 7.1 | 1.67 | 23.6 | 2.17 | 30.7 | | | | | | | | | 0.55 | |
| | | P | | 7.62 | 2.57 | 33.7 | 3.99 | 52 4 | 4 49 | 590 | 0.39 | 5.1 | 0 79 | 10.4 | 1.18 | 15.5 | 1.19 | 15.6 | | | | | | | 1.62 | 21.3 | 2.12 | 27.9 | 1.06 | 13.9 | 0.56 | 7 3 | 0.64 | 0.57 |
| INF. HEPATITIS | 6 | S | 7.70 | | 3 47 | 45.1 | 4.23 | 54 9 | | | 040 | 5.2 | 0 83 | 10.8 | 1.23 | 16.0 | 1.28 | 16.6 | 0.26 | 3.4 | 1.46 | 18.9 | 1.72 | 22.3 | | | | | | | | | 0.82 | |
| | | P | | 8.15 | 3 43 | 42.1 | 4.01 | 49 2 | 4.27 | 524 | 0.44 | 5.5 | 0.94 | 11.5 | 1.38 | 17.0 | 1.21 | 14.8 | | | | | | | 1.42 | 17.4 | 1.68 | 20.6 | 0.71 | 8.7 | 0.45 | 5.5 | 0.86 | 0.80 |
| TOXIC HEPATITIS | 6 | S | 7.24 | | 3 10 | 42.8 | 4.14 | 57.2 | | | 043 | 6.0 | 0.77 | 10.6 | 1.20 | 16.6 | 1.23 | 17.0 | 0.31 | 4.3 | 1.40 | 19.3 | 1.71 | 23.6 | | | | | | | | | 0.75 | |
| | | P | | 7.60 | 3.11 | 41.0 | 3.82 | 50 2 | 4.13 | 543 | 0.44 | 6.4 | 0.81 | 10.7 | 1.30 | 17.1 | 1.29 | 17.0 | | | | | | | 1.23 | 16.1 | 1.54 | 20.2 | 0.67 | 8.8 | 0.36 | 4 7 | 0 81 | 0.75 |
| OBST JAUNDICE | 3 | S | 7.20 | | 3 13 | 43 5 | 407 | 56.5 | | | 0 41 | 5.7 | 1.18 | 16.4 | 1.59 | 22.1 | 1.21 | 16.8 | 0.34 | 4.7 | 0.93 | 12.9 | 1.27 | 17.6 | | | | | | | | | 0.77 | |
| | | P | | 7 81 | 3.24 | 41 5 | 3 62 | 46.4 | 3.96 | 507 | 0.49 | 6.3 | 1 22 | 15.6 | 1.71 | 21.9 | 1.15 | 14.7 | | | | | | | 0.76 | 9.86 | 1 10 | 14.1 | 0.95 | 12.1 | 0 61 | 7.7 | 0.94 | 0.82 |
| MISC | 18 | S | 7.26 | | 3.64 | 50.1 | 3.62 | 49.9 | | | 0.45 | 6.3 | 0.74 | 10.2 | 1.19 | 16.5 | 1.01 | 14.7 | 0.21 | 2.9 | 1.15 | 15.8 | 1.36 | 18.7 | | | | | | | | | 0.96 | |
| | | P | | 770 | 3.62 | 47.0 | 3.43 | 44 5 | 3 62 | 47 3 | 0.44 | 5.7 | 0.86 | 11.1 | 1.29 | 16.8 | 1.12 | 14.5 | | | | | | | 1.02 | 13.2 | 1.23 | 15.8 | 0.65 | 8.5 | 0.44 | 5.7 | 1.06 | 1.00 |
| NORMALS | 10 | S | 7.32 | | 4.40 | 60 1 | 2.92 | 39.9 | | | 0.37 | 5 0 | 0.68 | 9.3 | 1.05 | 14.3 | 0.84 | 11.5 | 0.18 | 2.5 | 0.85 | 11.6 | 1.03 | 14.1 | | | | | | | | | 1 48 | |
| | | P | | 7.65 | 4.55 | 59.5 | 2.59 | 33.8 | 2.77 | 362 | 0.33 | 4.3 | 1 67 | 8.7 | 1.00 | 13.0 | 0.79 | 10.3 | | | | | | | 0.81 | 10.5 | 0.99 | 12.9 | 0.51 | 6.7 | 0.33 | 4.3 | 1.75 | 1 64 |

TABLE II

*Comparison of electrophoretic mobilities of protein components in serum and plasma*

| Diagnosis | No. of cases | Serum plasma | Av. alb. | Mobilities (cm²/volt sec. ×10⁻⁵) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | $\alpha_1$ | $\alpha_2$ | $\beta$ | $\phi$ | $\gamma_1$ | $\gamma_2$ | $\gamma_p$ (uncorr.) |
| Cirrhosis | 23 | S | 6.64 | 5.49 | 4.42 | 3.35 | — | 2.54 | 1.48 | — |
| | | P | 6.65 | 5.53 | 4.43 | 3.39 | 2.51 | — | — | 1.49 |
| Infectious hepatitis | 6 | S | 6.56 | 5.44 | 4.40 | 3.42 | — | 2.51 | 1.46 | — |
| | | P | 6.56 | 5.45 | 4.41 | 3.38 | 2.49 | — | — | 1.45 |
| Toxic hepatitis | 6 | S | 6.58 | 5.45 | 4.42 | 3.40 | — | 2.53 | 1.45 | — |
| | | P | 6.55 | 5.47 | 4.43 | 3.42 | 2.51 | — | — | 1.47 |
| Obstructive jaundice | 3 | S | 6.63 | 5.47 | 4.35 | 3.30 | — | 2.48 | 1.49 | — |
| | | P | 6.59 | 5.32 | 4.40 | 3.30 | 2.45 | — | — | 1.48 |
| Miscellaneous diseases | 18 | S | 6.64 | 5.47 | 4.40 | 3.41 | — | 2.54 | 1.50 | — |
| | | P | 6.62 | 5.44 | 4.41 | 3.38 | 2.49 | — | — | 1.48 |
| Normal | 10 | S | 6.66 | 5.48 | 4.43 | 3.43 | — | 2.59 | 1.58 | — |
| | | P | 6.63 | 5.46 | 4.45 | 3.46 | 2.54 | — | — | 1.58 |



Figs. 2a, b, c, d. Relation of Electrophoretic Serum and Plasma Albumins, Alpha Globulins, Beta Globulins and Albumin-Globulin Ratios

The decrease in the albumin component when compared to the values in the normal group was most marked in the cirrhotics, less in the other liver diseases, and least in the miscellaneous group. The cases of rheumatoid arthritis which formed most of the miscellaneous group are responsible for the albumin and globulin changes in this group and are similar to those found in liver disease though smaller. They are in agreement with those reported by others (16–18). All groups showed slightly higher serum than plasma values but these differences, both in percentage and absolute concentrations, were not significant. The correlation between serum and plasma albumin is good (Figure 2a) and the observed scatter is due largely to the inherent error in the electrophoretic procedure.

There was a tendency to slight increase of the alpha globulins in all the pathologic cases and most marked in obstructive jaundice (Table I) where the frequent association of lipids with alpha (as well as beta globulin) may account for this elevation. Elevated values of lipid were present in the three cases of obstructive jaundice. The alpha globulins were the only protein components in which the plasma values were greater than were the serum values, both relatively and absolutely. This was found to be true in all groups except the normal and cirrhotic groups. However, these differences between alpha globulins in serum and plasma were not significant. Figure 2b reveals that the spread on either side of the theoretical line of perfect correlation is greater than that of any of the other components. This was particularly true of the alpha$_2$ fraction.

Beta globulins (Table I) were increased in liver diseases as others have found (13–15). The relative percentage values in serum and plasma vary significantly but after they are converted to absolute values the variation loses its significance and does not exceed the limits of error of the method. Figure 2c reveals a good correlation between absolute values of beta globulin in serum and plasma.

The characteristically elevated gamma globulin in liver disease and rheumatoid arthritis is well illustrated in Tables I and III. This increase was most marked in cirrhosis, less so in hepatitis and least in obstructive jaundice and miscellaneous group. These findings are in agreement with those of previous authors (10–19). The normal serum gamma globulin values (average 14.1%) in this study are slightly higher than those reported by others (9, 20) because of our inclusion of the gamma$_1$ fractions of the total gamma values. This gamma$_1$ component normally forms approximately 2–3% of the total protein (2) and is often not included in the electrophoretically determined gamma globulin. The serum gamma globulins of all groups of cases studied including the normals were much higher than the plasma values (Tables I and III). Figure 1 reveals the cause of this significant discrepancy between serum and plasma gamma globulin values. The serum gamma globulin is composed of two fractions of different mobilities, a fraction labelled gamma$_2$ and a fraction with a greater mobility labelled gamma$_1$. This latter fraction has a mobility similar to fibrinogen and is therefore buried in the "fibrinogen" peak in the plasma pattern (Figure 1). Thus the apparent plasma gamma is the equivalent of the gamma$_2$ serum fraction (Tables I, III, Figures 1, 3). If the serum gamma$_1$ is determined in grams/100 ml. and this absolute value is then added to the absolute plasma gamma globulin value, the resultant corrected plasma gamma globulin value approaches the serum value. The difference between the serum gamma globulin and the corrected plasma gamma globulin then falls within the inherent error of the electrophoretic method (Tables I, III, Figures 3, 4). In cirrhosis this gamma$_1$ fraction is increased almost threefold from an average value of 2.5% in the normal group to 7.1%. There is a moderate increase in gamma$_1$ in the three obstructive jaundice cases. Only a slight increase in this component was present in the hepatitis cases and normal values were obtained in the miscellaneous group despite somewhat increased total gamma globulins in this latter group. After correction, the plasma total gamma globulin values were still lower than the serum values although the differences were much less than before correction. Prior to correction of

TABLE III

*Comparison of electrophoretic serum and plasma gamma globulins*

| | | % TOTAL PROTEIN | | | | gm/100ml | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| DIAGNOSIS | NO. CASES | $\gamma'$ | $\gamma^2$ | TOTAL $\gamma_s$ | $\gamma'$% OF Tot. $\gamma_s$ | $\gamma'$ | $\gamma^2$ | TOTAL $\gamma_s$ | TOTAL $\gamma_p$ UNCORRECTED | TOTAL $\gamma_p$ CORRECTED |
| CIRRHOSIS | 23 | 7.1 | 23.6 | 30.7 | 23.6 | 0.50 | 1.67 | 2.17 | 1.62 | 2.12 |
| RANGE | | (2.1 - 11.5) | (11.9-55.6) | (15.1 - 65) | (9.5 - 36.1) | | | | | |
| INF HEPATITIS | 6 | 3.4 | 18.9 | 22.3 | 15.2 | 0.26 | 1.46 | 1.72 | 1.42 | 1.68 |
| RANGE | | (2.3 - 4.5) | (14.8-22.0) | (16.9-27.5) | (12 - 20.3) | | | | | |
| TOXIC HEPATITIS | 6 | 4.3 | 19.3 | 23.6 | 18.6 | 0.31 | 1.40 | 1.71 | 1.23 | 1.54 |
| RANGE | | (1.8 - 6.0) | (10.7-40.0) | (12.5-44.9) | (10.9-34.4) | | | | | |
| OBST. JAUNDICE | 3 | 4.7 | 12.9 | 17.6 | 26.6 | 0.34 | 0.93 | 1.27 | 0.76 | 1.10 |
| RANGE | | (3.3 - 8.5) | (12.4-15.0) | (14 - 18.4) | (18.6-28.4) | | | | | |
| MISCELLANEOUS | 18 | 2.9 | 15.8 | 18.7 | 15.5 | 0.21 | 1.15 | 1.36 | 1.02 | 1.23 |
| RANGE | | (1.6 - 4.7) | (9.1-61.4) | (12.0-66.1) | (11.6-23.4) | | | | | |
| NORMAL | 10 | 2.5 | 11.5 | 14.1 | 17.7 | 0.18 | 0.85 | 1.03 | 0.81 | 0.99 |
| RANGE | | (1.1 - 3.4) | (8.5-17.2) | (12.6-19.1) | (10.0-17.3) | | | | | |

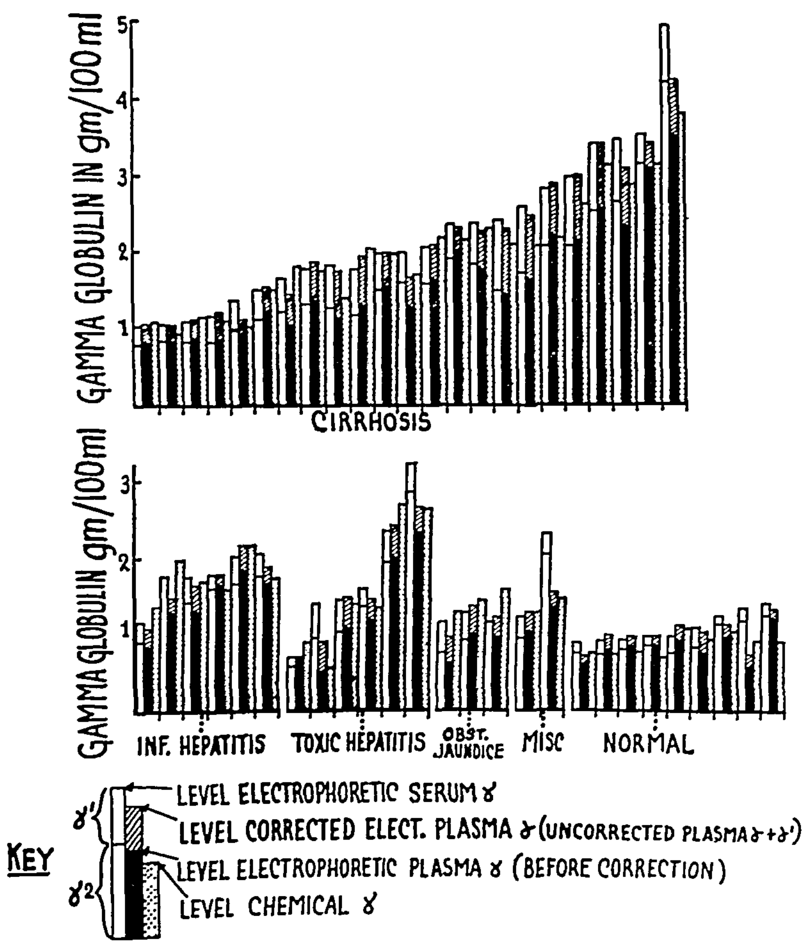


FIG. 3. RELATION OF ELECTROPHORETIC SERUM, PLASMA AND CHEMICAL GAMMA GLOBULINS

the plasma gamma globulin, particularly in the pathologic cases, the chemical gamma globulin values more closely resembled the serum electrophoretic results. After correction there was essentially no difference between the relation of the serum and plasma electrophoretic gamma globulin results and the chemical gamma globulin.

An analysis of fibrinogen data (Tables I, IV) reveals greatly elevated values in cirrhosis (average 13.9%, with a range between 6.07% and 28%). The obstructive jaundice cases also had elevated fibrinogen values and the other groups revealed smaller increases in this component. Figure 1 indicates the reason for these abnormal elevations in fibrinogen, particularly in cirrhosis where the plasma pattern contains a markedly elevated "fibrinogen" peak. In both the cirrhotic and normal electrophoretic patterns there is incomplete disappearance of the "fibrinogen" peaks in the corresponding serum patterns. In the normal pattern, a small plateau-shaped component is still present between the gamma and beta globulin complexes. In the cirrhotic pattern this remaining component is of much greater magnitude and accounts for almost 50% of the area underneath the plasma "fibrinogen" peak. This component, lying between the gamma and beta peak, is the gamma$_1$ globulin fraction of the serum and has electrophoretic mobility similar to fibrinogen. Inclusion of this gamma$_1$ fraction in the "fibrinogen peak" thus accounts for most of the marked fibrinogen elevations in the cirrhotics as well as the lesser elevations of this component in the other pathologic plasma specimens. The electrophoretic fibrinogen values can be corrected by subtracting from the originally elevated values, the absolute gamma$_1$ value as determined in the corresponding serum samples. The corrected values are much lower

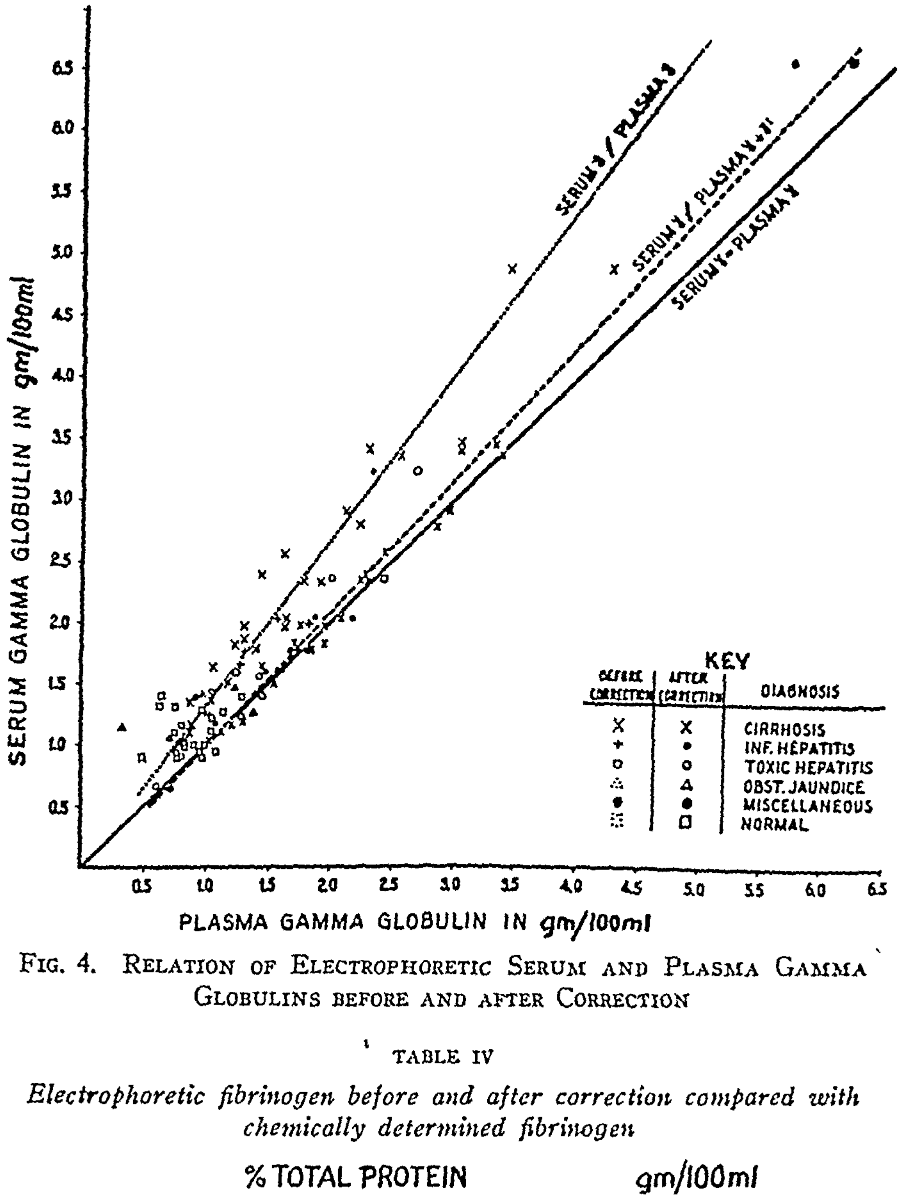


FIG. 4. RELATION OF ELECTROPHORETIC SERUM AND PLASMA GAMMA GLOBULINS BEFORE AND AFTER CORRECTION

TABLE IV

*Electrophoretic fibrinogen before and after correction compared with chemically determined fibrinogen*

| | % TOTAL PROTEIN | | | | gm/100ml | | | |
|---|---|---|---|---|---|---|---|---|
| DIAGNOSIS | NO. CASES | $\phi$ BEFORE CORRECTION | $\gamma'$ % OF T.P.p | CORRECTED $\phi$ | $\phi$ BEFORE CORRECTION | $\gamma'$ | CORRECTED ($\phi + \gamma'$) | CHEMICAL $\phi$ |
| CIRRHOSIS | 23 | 13.9<br>(6.0 – 28.0) | 6.6<br>(2.0 – 10.8) | 7.3<br>(3.0 – 20.5) | 1.06 | 0.50 | 0.56 | 0.34<br>(9 CASES) |
| INF. HEPATITIS | 6 | 8.7<br>(6.7 – 10.2) | 3.2<br>(2.3 – 4.0) | 5.5<br>(3.5 – 6.8) | 0.71 | 0.26 | 0.45 | 0.29<br>(4 CASES) |
| TOXIC HEPATITIS | 6 | 8.8<br>(5.9 – 11.1) | 4.1<br>(1.8 – 5.7) | 4.7<br>(1.7 – 7.2) | 0.67 | 0.31 | 0.36 | 0.25<br>(1 CASE) |
| OBST. JAUNDICE | 3 | 12.1<br>(10.8 – 12.4) | 4.4<br>(3.0 – 5.1) | 7.7<br>(5.7 – 9.4) | 0.95 | 0.34 | 0.61 | 0.55<br>(2 CASES) |
| MISCELLANEOUS | 18 | 8.5<br>(6.0 – 12.4) | 2.8<br>(1.6 – 4.4) | 5.7<br>(3.6 – 9.4) | 0.65 | 0.21 | 0.44 | 0.36<br>(1 CASE) |
| NORMAL | 10 | 6.7<br>(4.4 – 8.4) | 2.4<br>(1.6 – 3.3) | 4.3<br>(1.8 – 6.6) | 0.51 | 0.18 | 0.33 | 0.34<br>(10 CASES) |

than the original ones. The corrected absolute values approach the chemical values; in the normal group the electrophoretic results are still somewhat higher than those secured by the chemical method (Tables I, IV, Figure 5).

There is a good correlation between the albumin-globulin ratio as determined electrophoretically in the serum and plasma especially after the plasma total globulin has been corrected by the addition of gamma$_1$ globulin (Table I, Figure 2d).

In comparing the serum-plasma electrophoretic patterns, an unexpected finding was the fact that the greatest variations in the protein components were in the normals and this was true of all components.

## DISCUSSION

Electrophoretic analysis has been employed widely in recent years to study blood protein changes in liver disease (10–15, 19, 21). Usually this has been performed upon serum for the reasons previously mentioned. Dole (9) reported significant differences between serum and plasma protein electrophoretic values in a normal person primarily in the gamma and beta globulin fractions. Only the relative concentrations expressed in per cent of total protein were compared. Comparison of serum and plasma electrophoretic results are more meaningful if absolute rather than percentage values are compared. The percentage values are not strictly comparable, being fractions of total proteins which are dissimilar because of fibrinogen content in plasma. Chemical determinations may reveal similar values for total serum and plasma proteins because of dehydration of erythrocytes with consequent dilution of the plasma because of the anticoagulant (7, 8). Were it not for this dilution one would expect the fractions of total proteins to be about 4–5% greater in serum than in plasma with its fibrinogen (9). For a proper evaluation of any possible differences between serum and plasma electrophoretic values, the comparison should be made between absolute values determined from total serum and plasma proteins corrected for anticoagulant dilution of the plasma. Heparin alters the electrophoretic pattern (1). Theoretically, potassium oxalate might possibly cause changes due to chemical denaturation of the protein. However, in previous studies (22) we found no appreciable changes using standard amounts of potassium oxalate as an anticoagulant.

The albumin changes in liver disease and rheumatoid arthritis (Table I) were similar to those reported by others (10–19).

Reports in the literature differ as to the fate of alpha globulins in liver disease, some workers reporting irregular increases (13, 14) while others found no change (15, 21). Gutman (23) believes that there is an association between increased alpha globulin and decreased gamma globulin values. In this study the alpha globulins showed a slight increase in all the pathologic cases, the greatest increase being in obstructive jaundice. The elevated lipids on our cases of obstructive jaundice

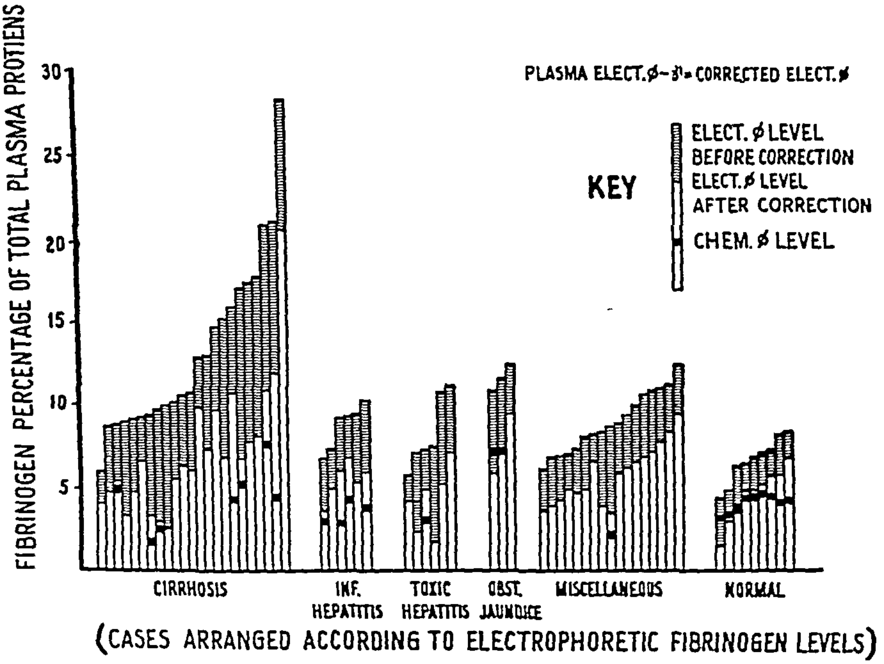


Fig. 5. Comparison of Electrophoretic Fibrinogen before and after Correction with Chemically Determined Fibrinogen

may account for this increase in alpha globulin. Lipids frequently may be associated with alpha as well as beta globulins (23). Dole (9) has suggested that the lower alpha values in serum as compared with plasma may be due to removal of this component in the clot. While the total serum alpha globulins in this study were less than that of plasma, it was not significantly so. However, as Table I reveals, serum alpha$_1$ globulin was lower than the corresponding plasma values while the reverse was true for the alpha$_2$ fraction. The greater spread on either side of the theoretical line of perfect correlation in this component (Figure 2b) also indicates a greater variation in this component than in any other protein fraction. The increased beta globulins in the liver diseases groups are in agreement with the findings of others (13–15, 24). There appears to be a significant variation in the relative percentage values of serum and plasma beta globulins. However, if these percentage values are converted to absolute values in grams/100 ml. that variation loses its significance and falls within the inherent limits of error of the method. The serum-plasma absolute values correlate well (Figure 2c). Dole (9), in comparing the serum and plasma of a normal individual, similarly found the beta serum value elevated and believed this to be due to overlapping of the beta globulin and "fibrinogen" peaks. However, he did not compare absolute values. When this was done in our cases it tended to nullify the serum and plasma differences.

The serum gamma globulin findings in the disease groups are in agreement with those recorded by previous authors (10–19, 21, 24). The significant difference between the serum-plasma gamma globulin values (Tables I and III), particularly in cirrhosis, is due to burial of the gamma$_1$ fractions in the "fibrinogen" peak of the plasma pattern. The greater the gamma$_1$ fraction the greater will be the serum-plasma difference in total gamma values. Electrophoresis, particularly in the pathologic cases, yields plasma gamma globulin values 15–20% lower than serum values. It is important, therefore, to determine whether gamma$_1$ globulin is increased in other diseases, lest misleading gamma globulin values be secured in plasma determinations in these diseases. The true picture of gamma globulin changes is found in serum determinations. The nature of gamma$_1$ globulin and the possible significance of its elevation in liver disease are discussed elsewhere (25).

An analysis of the reported observations on the plasma fibrinogen content in liver disease reveals a diversity of opinion (8, 11, 15, 21). The liver is probably the prime site of fibrinogen formation (26), and diminution of this protein has been reported in acute hepatic insufficiency such as in the damage experimentally produced by chloroform and other toxins (27). Diminished fibrinogen may also be found in humans in the severe hepatic insufficiency of acute yellow atrophy (8). Elevated fibrinogen values have been reported in cirrhosis (11, 21). Whitman and his co-workers (11) consider the possibility that the elevated "fibrinogen" peak in cirrhosis is due to an increase in gamma globulin as well as to an actual fibrinogen increase. Our data show this to be correct. The markedly elevated "fibrinogen" peaks often found in the cirrhotic electrophoretic pattern occur because the gamma$_1$ component has a similar mobility to that of fibrinogen and is concealed in the "fibrinogen" peak. Similarly, Zeldis and Alling (28) had noted spurious elevations in fibrinogen values in plasma from dogs and had attributed this to increments from adjacent components. Even after correction of the fibrinogen values by subtracting the amount contributed by the gamma$_1$ complex to the "fibrinogen" peak, the electrophoretic values for that component were still elevated above those determined chemically in the cirrhotic patients (Tables I, IV, Figure 5). Fibrinogen is the plasma protein which forms fibrin under the action of thrombin. Fibrinogen analysis by chemical means may give erroneously low values because of incomplete precipitation of fibrin or false high values due to occlusion of other plasma components to the clot (29*a*). The effect of variation of fibrinogen and thrombin concentrations and pH and of the presence of other proteins must be taken into consideration in the chemical determination of fibrinogen. The occlusion of other protein components, particularly lipoproteins and certain enzymes such as thrombin, is sufficient to introduce error into fibrinogen chemical analysis (29*b*). Fibrinogen is also more sensitive to thermal and chemical changes than are proteins (30). The question then arises as to whether the more reproducible method of electrophosesis, particularly after subtraction of gamma$_1$

globulin, is not a more accurate method of fibrinogen analysis than is the chemical method. Against such a supposition is the possibility of other protein moieties besides $gamma_1$ globulin having a similar mobility as fibrinogen. It is also questionable labeling an electrophoretic "fibrinogen" peak as fibrinogen in the sense of protein clottable with thrombin. Nevertheless, we believe that the difference between the electrophoretic and chemically determined fibrinogen indicates an actual increase of this component in cirrhotic liver disease.

The plasma electrophoretic pattern as such appears on inspection to be more characteristic of chronic liver disease than the serum electrophoretic pattern. In addition to the characteristically depressed albumin and elevated gamma globulin peaks seen in the cirrhotic serum pattern, there is also the markedly elevated "fibrinogen" peak present in the plasma electrophoretic pattern. We have not seen this triad of findings to such a marked degree in the electrophoretic pattern of any other pathologic condition. However, serum rather than plasma is preferable where it is desired to study the electrophoretic quantitative partition of the protein complexes in chronic liver disease. The elevation of $gamma_1$ globulin and its inclusion in the "fibrinogen" peak in the plasma pattern give a false impression of extreme fibrinogen elevation and at the same time cause the gamma globulin elevations to be lower than those found in serum. A more correct picture of the fibrinogen alterations in chronic liver disease requires simultaneous electrophoretic analyses on serum and plasma and correction for the increased $gamma_1$ globulin frequently associated with that pathologic state.

It is fully realized that the electrophoretic pattern, particularly in cirrhosis, may vary depending upon the stage and course of the disease. Our cases of cirrhosis varied from those in the inactive stage to those with marked liver damage. We were primarily interested not in the type of pattern in the different liver diseases nor in the changes taking place during the course of the disease, but in the comparison of the patterns in the serum and plasma determinations. Stage and course of disease had no effect upon these serum-plasma differences. In fact, the only significant differences were those in the gamma globulin and these did not depend upon the stage of disease but the type of disease. The $gamma_1$ globulin relation to hepatic damage as measured by the cephalin flocculation, thymol turbidity and zinc sulphate turbidity tests is shown in Figure 4 of the following article (25).

## SUMMARY

1. Paired serum and plasma samples of 66 persons with various hepatic or miscellaneous diseases and normal subjects were analyzed electrophoretically. The liver disease group included cases of cirrhosis, infectious and toxic hepatitis, and obstructive jaundice. The miscellaneous disease group was comprised of patients with rheumatoid arthritis, peptic ulcer, and multiple myeloma.

2. Serum protein changes in the various disease groups were similar to those previously reported by other authors.

3. Fibrinogen values were markedly elevated particularly in the cirrhotic group. This was due to the inclusion of a $gamma_1$ globulin fraction in the "fibrinogen" peak. This was also responsible for the serum gamma globulin being significantly greater than the plasma gamma globulin. The addition of $gamma_1$, as determined in the serum, to the total plasma gamma globulin yielded total gamma globulin values essentially similar to those of the serum. At the same time subtraction of $gamma_1$ from the elevated fibrinogen values, as revealed by the plasma patterns, reduced these values approximately by 35–45%. After this correction, there was still a significant difference between electrophoretic and chemically determined fibrinogen, indicating an actual increase of this component in cirrhotic liver disease. The $gamma_1$ burial in the "fibrinogen" peak was also responsible for the depressed values of total globulin and albumin/globulin ratio in plasma determinations. These likewise could be corrected for by addition of $gamma_1$ to the plasma globulin.

4. Aside from the significant serum-plasma difference in gamma globulin, the other protein components were essentially similar in serum and plasma, particularly when expressed as grams/100 ml. The beta globulin content in relative percentage was somewhat more elevated in serum than plasma. After conversion to absolute values however, this difference was not significant.

5. The routine use of plasma electrophoresis for the study of protein alterations in hepatic dis-

eases is not recommended. It is impossible to resolve and separate completely the components of the "fibrinogen" peak. Inaccurate gamma globulin and fibrinogen values result.

## ACKNOWLEDGMENT

The authors are indebted to Mrs. Jacqueline E. Budde for her assistance in the electrophoretic analyses and in the preparation of this manuscript.

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# ELECTROPHORETIC STUDIES IN LIVER DISEASE. II. GAMMA$_1$ GLOBULIN IN CHRONIC LIVER DISEASE[1, 2]

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(Submitted for publication January 5, 1951; accepted April 23, 1951)

The electrophoretic method of analysis has proved to be a most valuable aid in the study of protein alteration in hepatic diseases. Characteristic changes consisting of reduced albumin and increased gamma globulin components have been reported by many observers (1–3). The depressed albumin levels frequently observed in cirrhosis have been attributed to several factors, such as defective synthesis by the liver, inadequate dietary protein and the loss of albumin in ascitic fluid. There has been much speculation concerning the nature and cause of the globulin elevation as well as the fibrinogen changes in hepatic cirrhosis. At present the problem is still unsolved. Several observers have reported elevated electrophoretic fibrinogen values in cirrhosis (3, 4). One author (5) attributed some of this elevation to the migration of a gamma$_1$ globulin fraction in the mobility range of the "fibrinogen" peak. Deutsch and his co-workers (6) have described such a gamma$_1$ globulin component with a mobility similar to fibrinogen in normal serum. It constituted 2–3% of the total proteins. There are reports (7–9), of single cases of cirrhosis with a component in the electrophoretic pattern between the gamma and beta peaks which has been called the "X" component.

In a recent study (10) of paired serum and plasma from 66 persons having hepatic or miscellaneous diseases, and normal subjects, frequently great elevations of the "fibrinogen" peak were present in the plasma electrophoretic pattern. The serum patterns from the same persons did not show complete elimination of the "fibrinogen" peak but there was a component with a mobility between that of gamma and beta globulin. This component was included in the "fibrinogen" peak and accounted for a large portion of the elevation. It was present in normal sera, very high in cirrhotic sera, and there were smaller elevations in obstructive jaundice and acute hepatitis. The miscellaneous group had no increase of this globulin fraction. This component seems to be identical with the gamma$_1$ globulin component described by Deutsch in normals and the "X" component described in cirrhosis by others (7–9). It apparently is analogous to a new protein fraction found by many workers (11–15) in the serum of hyperimmunized animals and it, too, migrates between the gamma and beta globulin electrophoretic peaks. The present report deals with electrophoretic studies of the gamma$_1$ globulin alterations and their possible significance.

## MATERIAL AND METHODS

The subjects and methods were the same as those described in Part I of these studies, the preceding article (10), except for additional gamma$_1$ globulin determinations in serum alone in eight more cases of cirrhosis, four of infectious hepatitis, one of toxic hepatitis, five of obstructive jaundice, two of hemolytic jaundice, 73 of diabetes mellitus, 23 of poliomyelitis, 16 of rheumatoid arthritis and 21 of hypertrophic arthritis. Chemical globulin determinations were performed according to the method of Wolfson and Cohn (16). The method of Chaney (17) was used for the cholesterol determinations. In addition total lipids (18), zinc sulphate turbidity (18), cephalin flocculation (19) and thymol turbidity (20) were determined.

## RESULTS

The relation of gamma$_1$ globulin to the total gamma globulin in the serum and to the "fibrino-

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[1] A preliminary report of this investigation was included in the Proceedings of the Twenty-Second Annual Meeting of the Central Society for Clinical Research (Proc. Cent. Soc. Clin. Res., 1949, 22, 28).

[2] This investigation was supported in part by grants from the Commission on Liver Disease, Army Epidemiological Board, Office of the Surgeon General, U. S. Army, Washington, D. C. and from the Dr. Jerome D. Solomon Memorial Research Foundation, Chicago, Illinois.

[3] Solomon Fellow.

TABLE I

*Comparison of electrophoretic protein partition in a case of cirrhosis using different electrophoresed material*

| Electrophoresed material | Per cent of total proteins | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Alb. | $\alpha_1$ | $\alpha_2$ | $\beta$ | $\phi$ | $\phi$ (corr.) | $\gamma_1$ | $\gamma_2$ | Tot. $\gamma_s$ | Tot. $\gamma_p$ | Tot. $\gamma_p$ (corr.) |
| Serum | 45.6 | 2.5 | 2.8 | 22.0 | — | — | 4.0 | 23.1 | 27.1 | — | — |
| Serum and thrombin | 45.4 | 4.3 | 3.3 | 20.2 | — | — | 4.3 | 22.5 | 26.8 | — | — |
| Serum and oxalate | 44.4 | 4.7 | 5.2 | 17.7 | — | — | 4.4 | 23.6 | 28.0 | — | — |
| Plasma | 42.5 | 4.0 | 3.0 | 17.4 | 10.0 | 6.0 | — | — | — | 23.1 | 27.1 |

gen" peak in the plasma patterns is illustrated in Figures 1, 3, 4, and Tables I and III of the preceding article (10). The average normal value of the gamma$_1$ globulin in the group with paired serum and plasma samples was 2.5% of the total protein with a range between 1.7 and 3.4%. The cirrhotic patients had the most marked increase in this component, having an average gamma$_1$ globulin value of 7.1% with a range between 2.1 and 11.5% (Tables I and III of the preceding article [10]). In the group of cirrhotic cases in which only serum determinations were performed, the average gamma$_1$ was slightly higher (7.6%) with one case showing 19% gamma$_1$ globulin (Figure 1). The cases of obstructive jaundice and *acute hepatitis revealed less gamma$_1$ elevation.* In interpreting the results in these groups, particularly in the obstructive jaundice group, the limited number of cases must be considered. The average gamma$_1$ globulin values for the miscellaneous disease group was within normal limits. The total serum gamma globulin increases present in the cases of liver disease and rheumatoid arthritis are in agreement with previously reported observations (21–26). No relation existed between the gamma$_1$ globulin and the total serum proteins (Figure 2a). This is to be expected since the total serum proteins in the pathologic cases and the normal individuals are similar, whereas the gamma$_1$ fractions in these two groups are dissimilar. A relatively greater increase in the gamma$_1$ fraction was found in cirrhosis than in other liver diseases (Figure 2b). This increase is proportionately greater than the increase in total gamma globulin. The average gamma$_1$ globulin values for the miscellaneous group were normal despite the increase in total gamma globulin. Only two cases of rheumatoid arthritis in this group exhibited values beyond the normal range. The single case of multiple myeloma, despite the highest total gamma globulin percentage (60%), had a gamma$_1$ value of 4%, only slightly above the normal limit. The non-liver disease cases have average values of gamma$_1$ globulin within the normal range although in each group there were cases with elevated values (Figure 1). The gamma$_1$ increase is not merely a reflection of elevated total gamma globulin but apparently bears some relation to chronic liver disease although not necessarily confined to that pathologic state.

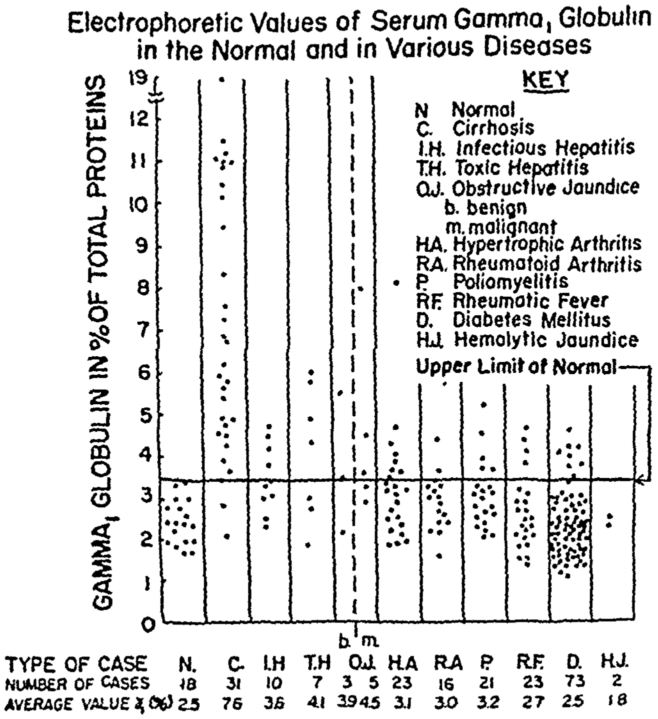

FIG. 1. ELECTROPHORETIC VALUES OF SERUM GAMMA GLOBULIN IN THE NORMAL AND IN VARIOUS DISEASES

To determine whether the gamma$_1$ globulin component was related to beta rather than gamma globulin, this component was compared with the electrophoretic beta globulin and the chemically-determined cholesterol and the total lipids which are said to be intimately related to the beta globu-

FIG. 2a

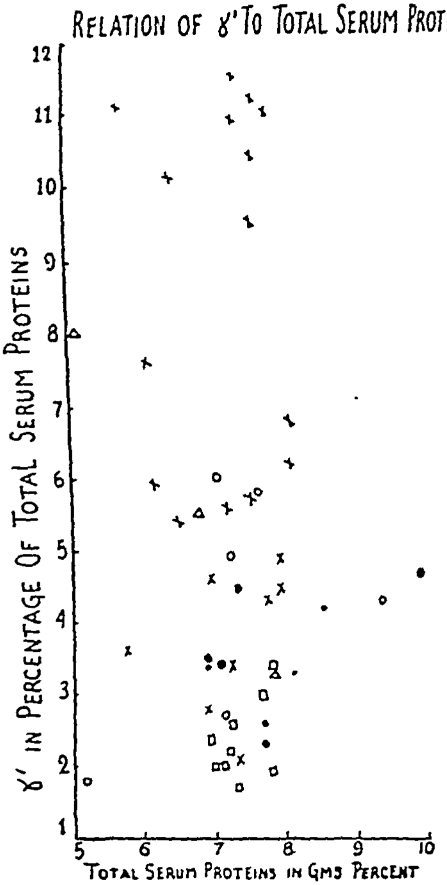

FIG. 2b

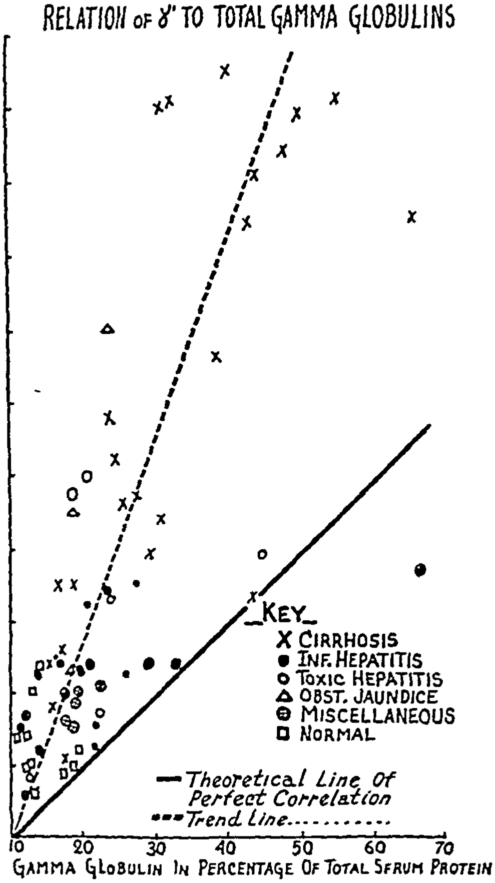

FIG. 2a. RELATION OF GAMMA$_1$ GLOBULIN TO TOTAL SERUM PROTEINS

FIG. 2b. RELATION OF GAMMA$_1$ GLOBULIN TO TOTAL SERUM GAMMA GLOBULINS

lins (27, 28). There is no relation between the gamma$_1$ component and the beta globulins, total cholesterol or total lipids (Figure 3). This is in keeping with Deutsch's contention that gamma$_1$ globulin is actually a gamma rather than a beta globulin. The similarity of all three scattergraphs, although showing no relation between gamma$_1$ globulin and beta globulin, cholesterol and total lipids, suggests that all but gamma$_1$ [illegible] pro-
[illegible] other. [illegible] is not a residual
[illegible] globulin does not [illegible] ma or beta globulin? What relation has it to other protein complexes situated between the gamma and beta components in the electrophoretic pattern in other diseases? When does it appear in human serum and what is its relation to similar electrophoretic components found in the serum of animals?

retic pattern was probably not residual fibrinogen nor could the serum-plasma electrophoreti-
ference be attributed to the anticoag
denaturation of protein fractions ltic gamma$_1$
relation of the gamma$_1$ component materials in
ogen in the plasma pattern ates that this com-
plasma [illegible] ma globulin difibrinogen nor was the
[illegible]coagulant respo of t for the serum-plasma electrophoretic differences. It is a protein component of normal human serum, designated gamma$_1$ by Deutsch who separated it from the bulk of the gamma fraction by means of ethanol fractionation (6). He found it to be present in normal human serum to the extent of 2–3% of the total serum proteins and that it was free of lipids and cholesterol. Our results are in agreement. Gamma$_1$ globulin is apparently the same protein fraction de-

gamma globulin values of plasma being less than those of serum. The comparison of the $gamma_1$ globulin values in normal subjects, cirrhotics and other disease states is illustrated in Figure 1. Elevations of $gamma_1$ globulin in other diseases, although present, are infrequent and are usually of mild degree only. In contrast the great majority of cirrhotics have $gamma_1$ globulin elevations and these are much higher than those found in other diseases.

## DISCUSSION

The characteristic alterations in the serum proteins in chronic liver disease, namely, hypoalbuminemia and hyperglobulinemia chiefly of the gamma fraction, have been reported by numerous observers (5, 21–23). Although failure of albumin synthesis by the liver is generally regarded as the cause of the marked hypoalbuminemia, other factors undoubtedly play a role. Inadequate protein intake, absorption or utilization and loss in ascitic fluid and urine may increase the albumin depletion in cirrhosis.

The cause of gamma globulin increase in chronic liver disease is obscure and has been the basis of much conjecture. The explanation frequently advanced that the hyperglobulinemia is compensatory for the lowered serum albumin seems improbable. This would not explain why the elevation is usually in the gamma fraction and why it frequently is sustained after the serum albumin has returned to normal. Albumin has a much higher osmotic activity than globulin and a rise in the latter probably would be of little significance in elevating a depressed colloid osmotic pressure resulting from hypoalbuminemia (5).

The perplexing problem of hyperglobulinemia in many diseases including cirrhosis is now further complicated by a necessity to explain the $gamma_1$ globulin fraction elevations characteristic of cir- [illegible] flection of elevated [illegible] parently bears some [illegible] although not necessarily [illegible] logic state.

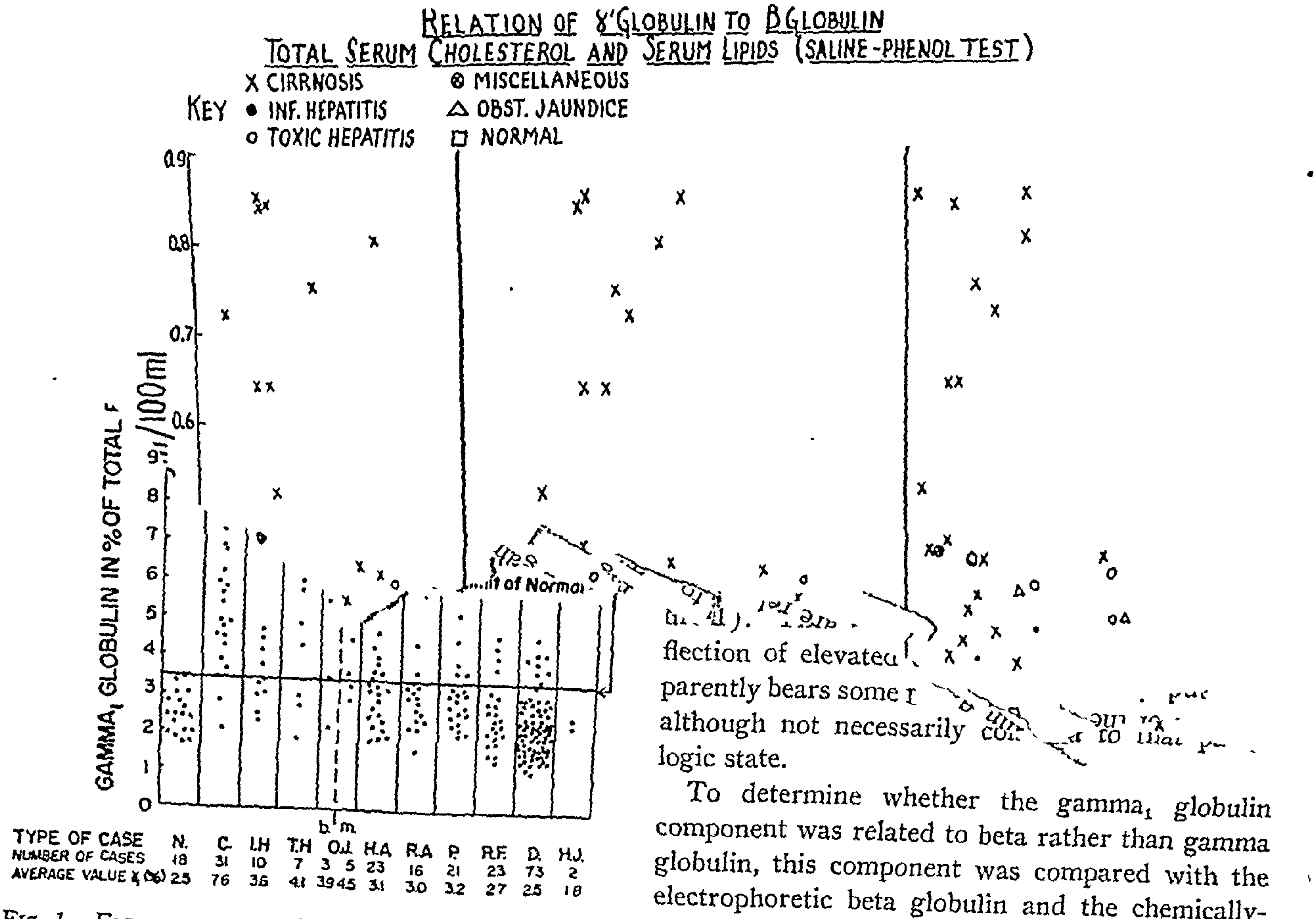

FIG. 1. ELECTROPHORETIC VALUES OF SERUM $GAMMA_1$ GLOBULIN IN THE NORMAL AND IN VARIOUS DISEASES

To determine whether the $gamma_1$ globulin component was related to beta rather than gamma globulin, this component was compared with the electrophoretic beta globulin and the chemically-determined cholesterol and the total lipids which are said to be intimately related to the beta globu-

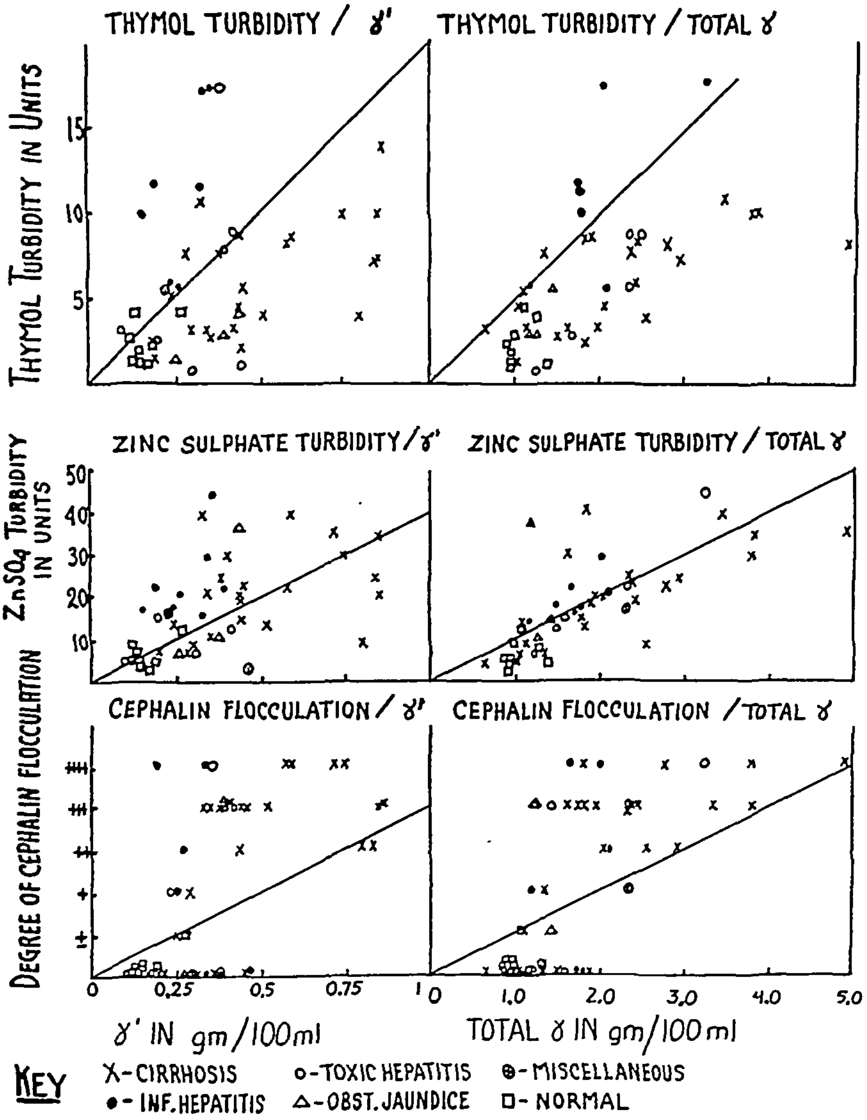

FIG. 4. RELATION OF GAMMA₁ GLOBULIN AND TOTAL SERUM GLOBULINS TO CEPHALIN FLOCCULATION, ZINC SULPHATE AND THYMOL TURBIDITY TESTS

rhosis. Before hypothesizing as to its origin, several questions concerning its nature must be answered. Is this component, found in the serum, merely residual fibrinogen which has not been completely removed in the clotting process? Is gamma₁ globulin denatured serum protein produced by the anticoagulant? If it is not a residual denatured protein, is it a gamma or beta globulin? What relation has it to other protein complexes situated between the gamma and beta components in the electrophoretic pattern in other diseases? When does it appear in human serum and what is its relation to similar electrophoretic components found in the serum of animals?

The similarity of the electrophoretic gamma₁ values of the different electrophoresed materials in the same patient (Table I) indicates that this component was not residual fibrinogen nor was the anticoagulant responsible for the serum-plasma electrophoretic differences. It is a protein component of normal human serum, designated gamma₁ by Deutsch who separated it from the bulk of the gamma fraction by means of ethanol fractionation (6). He found it to be present in normal human serum to the extent of 2–3% of the total serum proteins and that it was free of lipids and cholesterol. Our results are in agreement. Gamma₁ globulin is apparently the same protein fraction described in cirrhosis as the "X" component by some observers (7–9).

Gutman and his co-workers (29) and others (8, 30) have found in multiple myeloma an abnormal serum protein component with electrophoretic mobility between that of gamma and beta globulins. This abnormal component does not appear to be the gamma$_1$ globulin in the electrophoretic pattern of the cirrhotic. The former appears as a very tall narrow peak whereas the gamma$_1$ globulin component is not as narrow and tall and is in the form of a plateau rather than a distinct peak.

Purified Wassermann antibody also migrates with a mobility intermediate between beta and gamma globulins (31). However, the sera of syphilitic patients show no electrophoretic changes referable to this specific antibody. There is also no correlation between the titer of the quantitative Kahn reaction and increase in gamma globulin (32, 33). The concentrations of antibody are too low for detection by electrophoresis.

Our data reveal gamma$_1$ elevations infrequently in pathologic states other than liver disease. Moreover when they occur they are usually of slight degree only. In contrast the great majority of cirrhotics in our series had gamma$_1$ elevations and they were much higher than those found in sporadic cases of other types of disease showing elevations. Wallis (34) described a component with an electrophoretic mobility between that of gamma and beta globulins in three out of four cases of rheumatoid arthritis. He called this a "T" component and found that it comprised from 4.9 to 8.9% of the total serum proteins in these cases of rheumatoid arthritis. This "T" component, similar to the "X" component described in cirrhosis (7–9), is identical with the gamma$_1$ globulin in our cases. However, such elevations were present in only two of our 27 cases of rheumatoid arthritis and their elevations were only of very meager degree. The elevation of the gamma$_1$ component in human serum, although not pathognomonic, is highly characteristic of chronic liver disease.

What causes the gamma$_1$ elevation in cirrhosis? Has it any relation to the increase in total gamma globulin in this disease, the cause of which is conjectural? The intimate relation of antibodies and gamma globulin has long been known (11–15, 35–37). The increase in globulin in many infections consists not only in an elevation of specific antibodies identified by immunochemical methods but also in an increase that has been called "nonspecific antibody" or "reaction" globulin (38). Are these analogous to the gamma$_1$ and gamma$_2$ globulins? Animal experimentation has formed the basis for most of our knowledge of antibody formation. The antibodies of animals from various species immunized with different antigens are present in the globulin fraction having an electrophoretic mobility either of gamma globulin or between that of gamma and beta globulin. This latter component, not present normally in animals (6, 11–15), has been designated beta$_2$ by Kekwick and Record (15) and as "T" component by Van der Scheer and Wyckoff (12, 14). It has different physical and chemical properties from the classical gamma globulin and apparently is identical with the globulin fraction in normal human serum designated gamma$_1$ by Deutsch and associates (6) who showed that in normal persons this fraction was associated with a variety of antibodies. This gamma$_1$ globulin which was similarly found present in our normal subjects was elevated in most of our cirrhotic cases. Can the gamma$_1$ elevation in chronic liver disease be attributed to antibody formation?

The gamma$_1$ elevations in our subjects were most marked in the chronic liver cases. Elevations in the obstructive jaundice group may have been due to the secondary parenchymal damage revealed by liver function studies in these cases. It is impossible to draw any conclusions from only three cases. The elevation of the gamma$_1$ globulin component in chronic liver disease and the slight elevation in acute types of hepatic damage might appear to suggest typical antibody response such as is seen in infections in which antibodies increase as the condition becomes chronic. However, previous studies (39) have shown that scarring following viral hepatitis may occur with decreasing concentrations of electrophoretically separated gamma globulin. Viral hepatitis, the only hepatic condition in this study definitely known to be infectious, strangely enough showed little [illegible] gamma$_1$ fraction. On the other hand, the cases of cirrhosis of the Laennec's type in which an infectious agent is not usually implicated showed the greatest elevation of gamma$_1$ globulin. We compared the gamma$_1$ globulin in cases of alcoholic cirrhosis (in which malnutrition was a major factor) with that found in several cases of cirrhosis of the Hanot and cholangiolitic types presumed to be secondary to some form of infection such as viral hepatitis. There was essentially no difference in gamma$_1$ alteration in the "malnutrition" and "infectious" types of cirrhosis. Malnutrition experiments in human (40, 41) and animals (42) have shown that malnutrition is not responsible for any rise in gamma globulin. We must therefore look to some other cause than infection or malnutrition for the elevation of the gamma globulin and particularly the gamma$_1$ portion which in hyperimmunized animals (11–15) and normal humans (6) is rich in antibodies.

Certain features of the cirrhotic state itself lend support to a possible relation between gamma globulin increase and antibody formation in cirrhosis. Cirrhosis is frequently featured by an increased mesenchymal reaction. Increase in lymphocytes and plasma cell elements (43) may be striking in the bone marrow (44). The degree of plasma cell and lymphocyte hyperplasia correlates roughly with the degree of hyperglobulinemia (44, 45). Berlin and his co-workers (46) could not confirm these findings. They state, however, that where hyperglobulinemia exists without plasmacytosis in the bone marrow, other sites of proliferation of plasma cells must be considered, especially the liver and spleen or specific sites of chronic infection. They state that it is possible that globulins may be formed at several different sites, namely, in plasma cells, lymphocytes and reticulo-endothelial cells. Histiocytic proliferation in the liver likewise has been reported to be related to a reversal in the albumin-globulin ratio (47). Pulp hyperplasia, consisting largely of proliferation of active histiocytes in the lumen of the sinuses (48), is frequently a factor in the splenomegaly of cirrhosis. Bjørneboe and Gormsen (49) immunized rabbits with polyvalent vaccine which resulted in plasma cell proliferation in many organs, especially in the spleen and liver; the degree of this plasma cell proliferation closely corresponded to the antibody concentrations. They believed that the antibodies were formed by plasma cells and the increase in splenic weight after intensive immunization was due to the plasma cell proliferation in the spleen. The relation between hyperglobulinemia and plasma cell proliferation and the possible role of the histiocytes as globulin producers has been mentioned by other authors (50–53). The hyperglobulinemia may well be related to the histiocytosis and plasma cell activity frequently found in the liver, spleen and bone marrow in cirrhosis.

Since infections have not been proved as a causative factor in most cases of Laennec's cirrhosis, we must look for some other cause of the increased mesenchymal and globulin reactions in the cirrhotic state. Marked increases in plasma gamma globulin occur frequently in heterogenous diseases having in common extensive tissue breakdown such as in chronic suppurative infections (5). Gutman believes that the mechanism here may be homologous tissue antibody formation. It is conceivable that liver cell breakdown products of a protein nature act as an autogenous antigen causing a mesenchymal proliferation in the liver, spleen and bone marrow with a consequent or concomitant rise in gamma globulin. Necrosis of parenchymal cells may lead to fibrous tissue proliferations seen in cirrhosis (54).

The reason for the more frequent and greater elevation of gamma$_1$ globulin in chronic liver disease as compared with other diseases may be the damaged liver's inability to metabolize protein-breakdown products. This allows them to circulate and possibly act as autogenous antigens.

We have described the marked increase of gamma$_1$ globulin in cirrhosis and have hypothesized its nature and the reason for its presence in chronic liver disease. The gamma$_1$ globulin component is normally present in human blood and has been shown by Deutsch to have antibody activity (6). In addition, many workers have definitely demonstrated in animals an analogous protein component which is not present normally, but arises only after hyperimmunization with various bacterial antigens. Thus gamma$_1$ globulin in normal man and in hyperimmunized animals has been shown to have antibody properties. There is a possibility that circulating protein breakdown products, not metabolized by the diseased liver, act as an autogenous antigen and are responsible for the increased histiocytic plasma cell activity and increased gamma$_1$ globulin content frequently found in the cirrhotic state.

Realizing that this report has raised more questions than it has solved, we nevertheless believe that the approach to the complex problem of gamma globulin alterations in liver disease is promising and warrants further investigation using electrophoretic and immunochemical methods.

## SUMMARY

1. Electrophoretic determinations were performed on the sera of 241 persons, including 18 normal, 56 patients with various liver diseases and 167 patients with miscellaneous diseases.

2. There were marked elevations of a gamma$_1$ component between the commonly described gamma and beta globulin in the electrophoretic pattern in cirrhosis, and smaller elevations in other liver disease.

3. Average values in conditions other than liver disease were within the normal range.

4. The significance of this gamma$_1$ elevation in chronic liver disease and its possible relation to antibody formation are discussed.

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# HYPONATREMIA IN HEPATIC CIRRHOSIS FOLLOWING PARACENTESIS[1]

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(Submitted for publication January 15, 1951; accepted April 23, 1951)

The retention of water without a physiologically equivalent amount of sodium following abdominal paracentesis has been studied in two patients with advanced cirrhosis of the liver. In each there developed manifestations considered characteristic of sodium deficit, although there was no change in the total body sodium at the time these appeared. Such retention of water in excess of salt, regularly observed when large external losses of salt and water are replaced with water alone (1–3), has been noted in certain cases of heart failure and chronic renal disease (4–6) as well as in decompensated hepatic cirrhosis (7, 8). Whether the retention is primarily mediated through renal hemodynamic changes, altered tubular function, or increased antidiuretic activity is not clear. Observations concerning the development of this condition in two patients with cirrhosis are recorded below.

## METHODS

During the periods of study both patients were offered diets calculated to contain 100 grams each of protein and fat, 300 grams of carbohydrate, approximately 100 mEq. of potassium and no more than 15 mEq. of sodium. Dietary rejections were measured. Free access to water was allowed except when otherwise noted. The patients were weighed daily and the volume of urine collected during each 24 hour period was measured. The volume of fluid taken orally was estimated from conventional household measures. Venous blood samples were obtained daily in the post-absorptive state, without stasis in the first patient. Stasis was usually unavoidable in the second patient so that determinations of hemoglobin, hematocrit and serum protein were considered unreliable. The volume of packed red blood cells was measured in Wintrobe tubes (9); hemoglobin photometrically (10); serum total proteins by micro-Kjeldahl (11) and by gradient tube (12); chloride by Hald's modification of the Patterson micro-method (13); creatinine by Peters' modification of the Folin procedure (14); non-protein nitrogen by micro-Kjeldahl with Nesslerization (15); and sodium and potassium by means of the Barclay internal standard flame photometer. Except where otherwise noted, urine was collected over 24 hour periods and was analyzed for chloride by the Volhard-Harvey method (16), for total nitrogen by the micro-Kjeldahl procedure (11), and for creatinine, sodium, and potassium by the methods employed for serum. Change in total body water (liters) was taken to equal change in weight (kilograms).

## CASE REPORTS AND RESULTS

### *Case I.*

W. T. (CVAH No. 20063), a 58 year old white male, had been a chronic alcoholic for many years. One year before admission he developed ascites and jaundice at which time a low sodium diet was prescribed. He received frequent injections of mercurial diuretics but paracentesis was required for the relief of ascites, first monthly, later at two-week intervals. Following each paracentesis, disturbance of the sensorium of several days duration was regularly observed by the attending physician. Two days prior to the first hospitalization, paracentesis was performed and a mercurial diuretic was given. The patient also received 0.2 gram of sodium pentobarbital. Within a few hours he became hyperactive, confused, and vomited several times. At about this time he complained of cramps in his legs.

On admission (October 15, 1949) he appeared poorly nourished, acutely and chronically ill and was euphoric, confused, and slightly jaundiced. A few spider angiomata were present. The abdomen revealed signs of moderate ascites with a firm non-tender liver edge at the costal margin. The spleen was palpable 6 cm. below the costal margin. Peripheral and sacral edema were absent. Tests of liver function were consistent with marked hepatocellular disease. There was a moderate normochromic anemia. The specific gravity of the urine was 1.029. No abnormalities were noted on routine urinalysis. Renal excretion of phenolsulfonphthalein was 64 per cent in two hours. The blood non-protein nitrogen was 42 mg. per 100 ml. The concentration of chloride in the serum was 92 mEq. per liter. Several grams of sodium chloride were given shortly after admission. The muscle cramps promptly disappeared and the mental confusion cleared rapidly. The serum chloride rose to 100 mEq. per liter and the NPN fell to 29 mg. per 100 ml. The patient was discharged 11 days after admission much improved, but with persistent moderate ascites.

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[1] Reviewed in the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions published by the authors are the result of their own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

Abdominal paracentesis was again performed 16 days after discharge by the family physician and a week later, November 19th, the patient was readmitted in a state of confusion with clinical and laboratory findings as before. Without specific treatment he became oriented within 24 hours. Another paracentesis, performed in the hospital on December 3rd, was followed by confusion. He improved promptly when sodium chloride was given.

Detailed studies were carried out from December 14th to December 31st. (See Table I.) On December 15th, the serum sodium was 130 and chloride, 103.9 mEq. per liter. $CO_2$ content was 18 mEq. per liter and showed no significant change throughout the study. The hematocrit was 30 per cent. Following the removal of 8.6 liters of ascitic fluid the weight was 58.17 kilograms. The patient became thirsty and ingested considerable water. Forty-four hours later his weight had increased by 1.95 kilograms, the concentration of sodium in serum had fallen to 125 and that of chloride to 98.7 mEq. per liter, with the hematocrit rising to 34 per cent. Apathy, weakness, confusion, nausea and vomiting appeared. The blood pressure fell, with narrowing of the pulse pressure; marked tachycardia developed. The urine which had been scanty throughout was further suppressed prior to therapy. The serum non-protein nitrogen, consistently 40 mg. per 100 ml. or less during the previous seven days, rose to 47; the serum creatinine, consistently below 1.09 mg. per 100 ml., rose to 1.22 on December 17th. On this date 188 mEq. of sodium chloride were given intravenously as a 3 per cent solution. The next day the patient remained confused and apathetic with continued hypotension and tachycardia. Although serum sodium was now 131.5 mEq. per liter, the hematocrit continued to rise to 37 per cent. Accordingly, an additional 402 mEq. of sodium chloride in 3 per cent solution, and 50 grams of concentrated human albumin were administered intravenously. On the following day clinical improvement was striking. The serum sodium was 138 mEq. per liter. The hematocrit had returned to 30 per cent.

The patient was then maintained on a regular diet without salt restriction. On December 26th, 10.3 liters of ascites were removed. The patient was requested to limit his fluid intake. Nevertheless, because of intense thirst, he drank large quantities of water. Sodium chloride was given in proportion of approximately 135 mEq. to each liter of water retained; 550 mEq. the day of paracentesis and 426 the following day. On this program the concentrations of sodium and chloride in serum remained normal and there was no evidence of hemoconcentration or nitrogen retention. The sensorium remained clear. No signs of circulatory impairment developed. Some nausea did occur but this may have been related to ingestion of salt tablets and to abdominal distention associated with the accelerated rate of reaccumulation of ascites.

The retention of water and sodium for the two periods described are represented in the table and Figure 1. During the first period water retention was large although the intake of sodium was initially very small. When symptoms developed 44 hours after the first paracentesis, hypertonic sodium chloride was required to restore the body water to relatively normal tonicity. During the second period, when very large amounts of sodium chloride were given, the patient reaccumulated ascites at a very rapid rate and again required paracentesis of 11.5 liters of fluid on December 30th. It is of interest that on the second day of this regime the urine contained 54 mEq. of sodium whereas the excretion was less than 1 mEq. on all other days.

On December 31st, the patient developed bronchopneumonia, from which time on his condition deteriorated rapidly. Enough sodium chloride was given each day to render the fluid intake approximately isotonic. The concentration of sodium in serum remained normal throughout the remainder of his course and no symptoms suggestive of salt depletion appeared. However, he gradually lapsed into coma with increasing jaundice, and despite apparently successful therapy of the pneumonitis with antibiotics, death in hepatic coma occurred on January 14, 1950.

Post-mortem examination confirmed the clinical diagnosis of advanced portal cirrhosis with esophageal varices.

### *Case II.*

W. F. (CVAH No. 23530), a 58 year old white male, had consumed moderate quantities of alcohol for 25 years prior to hospitalization on May 3, 1950. He had noted anorexia and weight loss nine months before admission; two months later his abdomen began to swell. Three months prior to admission a paracentesis was performed; he was placed on a salt poor diet and received mercurial diuretics. Paracenteses were required at intervals of four to six weeks and were followed on each occasion by weakness and general malaise for two or three days unassociated with muscle cramps, disturbed sensorium or gastrointestinal symptoms.

On admission he appeared malnourished. The extremities were wasted and the abdomen was markedly distended. He was mentally alert. Numerous spider angiomata were present. Examination of the lungs and cardiovascular system revealed no abnormalities except for mild tachycardia and a blood pressure of 150/88. Numerous dilated superficial veins were present over the distended abdomen and frank signs of fluid were present. After the removal of ascitic fluid the edge of a firm non-tender liver could be felt 4 cm. below the costal margin. The spleen was not felt. There was no subcutaneous edema.

A mild hypochromic anemia was present. Urinalysis was unremarkable except for a specific gravity of only 1020 despite marked oliguria. Tests of liver function indicated marked hepatocellular damage, although bilirubin levels were normal. $CO_2$ content was 20.4 to 23.4 mEq. per liter during the period of study. Other data, recorded in the table, indicated moderate impairment of renal function. X-ray examinations failed to demonstrate esophageal varices but revealed a benign appearing ulcer crater on the lesser curvature of the stomach.

Shortly after admission the diet previously described, containing approximately 15 mEq. of sodium, was given.

TABLE I

*Analyses of blood and serum, exchanges of water and sodium, and creatinine clearance before and after paracentesis*

| Subject | Period | Weight* | Serum* |  |  |  |  |  | Blood* |  | Intake |  | Urinary output |  |  |  | Na balance | Change in total body water† | Ascitic fluid removed |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
|  |  |  | Na | K | Cl | Total protein | Creat. | NPN | Hct | Hb | Volume | Na | Volume | Na | Creat. | Creat. clear. (24 hr.) |  |  |  |
|  | *date* | *kg* | *mEq/L* | *mEq/L* | *mEq/L* | *grams/ 100 ml* | *mg/ 100 ml* | *mg/ 100 ml* | *%* | *grams/ 100 ml* | *L* | *mEq* | *ml* | *mEq* | *mg* | *ml/min* | *mEq* | *L* | *L* |
| W. T. (Case I) | 12/14–15 | 65.36 | 133.0 | 5.1 | 107.5 | 5.7 | 1.05 | 39 | 31.7 | 9.9 | 2.0 | 3.5 | 685 | 0.5 | 769 | 51 | + 3 | +0.53 |  |
|  | 12/15–16 | 65.89 | 130.0 |  | 103.9 | 5.7 | 1.02 | 40 | 30.0 | 9.6 | 1.7 | 4.0 | 860 | 0.3 | 1059 | 72 | + 4 | +1.40 |  |
|  |  | 58.17‡ |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 8.6 |
|  | 12/16–17 | 58.69 | 130.5 | 6.0 | 102.5 | 4.9 | 1.03 | 39 | 32.0 | 9.9 | 2.2 | 1.5 | 560 | 0.1 | 724 | 44 | + 2 | +1.43 |  |
|  | 12/17–18 | 60.12 | 125.0 | 5.7 | 98.7 | 5.0 | 1.22 | 47 | 34.0 | 10.2 | 3.8 | 188.0 | 1030 | 0.7 | 890 | 52 | +187 | +0.63¶ |  |
|  | 12/18–19 |  | 131.5 | 5.3 | 100.3 |  | 1.17 | 38 | 37.0 | 11.2 | 2.7 | 432.0 | 430 | 0.3 | 881 | 54 | +432 | +0.63¶ |  |
|  | 12/19–20 |  | 138.0 | 5.2 | 108.4 | 4.8 | 1.12 | 35 | 30.2 | 9.7 | 2.8 | 200 + | 485 | 0.3 | 851 | 55 |  | +0.63¶ |  |
|  | 12/20 | 62.00 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 12/26–27 | 72.50 | 136.2 |  | 109.6 | 5.0 | 1.07 | 39 | 29.0 | 8.7 | 5.4 | 550§ | 365 | 0.2 | 860 | 53 | +550** | +4.58 |  |
|  |  | 61.92‡ |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 10.3 |
|  | 12/27–28 | 66.74 | 133.3 |  | 111.9 | 4.4 | 0.99 | 36 | 27.0 | 8.4 | 4.0 | 426 | 720 | 53.9 | 1164 | 80 | +372 | +1.97 |  |
|  | 12/28–29 | 68.71 | 135.4 | 4.6 | 106.7 | 5.0 | 1.04 | 41 | 28.9 | 8.9 | 2.6 | 177 | 325 | 0.2 | 709 | 52 | +177 | +2.26 |  |
|  | 12/29–30 | 70.97 | 132.2 | 4.8 | 106.5 | 5.0 | 0.87 | 40 | 28.9 | 9.1 | 3.1 | 210 | 690 | 0.3 | 793 | 62 | +210 | +1.10 |  |
|  | 12/30–31 | 72.07 | 135.7 |  | 107.1 | 5.1 | 0.91 | 34 | 30.0 | 9.1 | 4.4 | 464 | 530 | 0.9 | 751 | 56 | +463 |  | 11.5 |
| W. F. (Case II) | 5/6–7 | 78.24 | 134.8 | 5.9 | 105.0 | 6.4 | 1.27 | 44 |  | 11.3 | 2.1 | 15 | 775 | 0.5 | 1308 | 69 | + 14 | +1.53 |  |
|  | 5/7–8 | 79.77 | 133.5 | 5.8 | 100.4 | 6.6 | 1.36 | 43 |  |  | 1.7 | 14 | 370‖ | 0.2‖ | 522‖ |  | + 14‖ | +0.38 |  |
|  | 5/8–9 | 80.15 | 133.2 | 5.7 | 101.6 | 6.3 | 1.33 | 44 |  | 11.7 | 2.4 | 15 | 815 | 0.5 | 1137 | 57 | + 14 | +0.89 |  |
|  | 5/9–10 | 81.04 | 129.8 | 6.4 | 100.1 | 6.3 | 1.44 | 43 |  | 11.4 | 1.8 | 9 | 655 | 0.3 | 835 | 36 | + 9 | +0.21 |  |
|  |  | 64.66‡ |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 16.4 |
|  | 5/10–11 | 64.85 | 125.0 | 6.4 | 96.1 | 5.7 | 1.78 | 46 |  | 12.3 | 1.0 | 9 | 675 | 0.3 | 841 | 33 | + 9 | +0.49 |  |
|  | 5/11–12 | 65.34 | 124.6 | 6.7 | 92.6 | 5.8 | 1.74 | 54 |  | 11.8 | 1.8 | 207 | 890 | 0.4 | 966 | 37 | +207 | +0.39 |  |
|  | 5/12–13 | 65.73 | 128.0 | 7.0 | 100.3 | 5.8 | 1.77 | 53 |  | 11.7 | 1.1 | 89 | 530 | 0.3 | 672 | 27 | + 89 | +0.33 |  |
|  | 5/13–14 | 66.06 | 126.3 | 6.8 | 96.9 | 6.0 | 1.90 | 54 |  | 11.7 | 1.2 | 41 | 810 | 0.4 | 945 | 35 |  |  |  |

* At beginning of period.
† Exclusive of ascitic fluid removed.
‡ After paracentesis.
§ Exclusive of dietary intake.
‖ Incomplete collection.
¶ Mean daily increment 12/17 to 12/20.
** Exclusive of dietary intake.

Potassium and nitrogen excretion closely paralleled calculated intake. Chloride excretion and calculated balance approximated the values for sodium. These data have accordingly been omitted from the table.

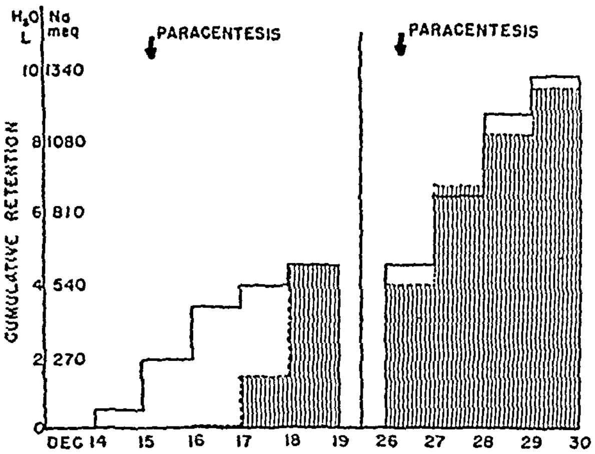

FIG. 1. CASE I. CUMULATIVE RETENTION OF TOTAL BODY WATER AND SODIUM

Ordinate scale constructed to represent 135 mEq. of sodium equivalent to 1.0 liter of water. Dotted line above hatched area represents retained sodium. Solid line represents water retained. Open area represents water retained in excess of sodium.

From May 6th to May 9th, before paracentesis, his weight increased almost 3 kilograms (Table I). During this period the concentration of sodium in serum fell from 134.8 to 129.8 mEq. per liter and that of chloride from 105.0 to 100.1 while serum potassium rose from 5.9 to 6.4 mEq. per liter. On May 9th, 16.4 liters of ascitic fluid were removed by paracentesis. The next morning, the concentrations of sodium and chloride in serum were 125.0 and 96.1 mEq. per liter respectively. On the morning of May 11th, the serum chloride had fallen further to 92.6 mEq. per liter, while the concentration of sodium appeared to have remained essentially unchanged and carbon dioxide content increased by 2 mEq. per liter. Serum potassium had increased to 6.7 mEq. per liter and the non-protein nitrogen to 56 mg. per 100 ml. However, the patient remained quite comfortable and ate well. During the afternoon increasing weakness and fatigue were noted together with some nausea. The patient refused supper and at midnight complained of severe nausea and persistent hiccoughs. He was distressed and apprehensive but the sensorium was clear and no muscle cramps developed. The pulse rate rose from 80 to 106 with a fall in blood pressure from 120/80 to 95/80. This deterioration of the clinical condition was unassociated with any further change in the concentration of sodium or chloride in serum, but by midnight, 60 hours after the paracentesis, the potassium rose even higher to 7.4 mEq. per liter. He was given 200 mEq. of sodium chloride as a 3 per cent solution intravenously at this time. During the next eight hours nausea gradually disappeared and he became much more comfortable. The concentration of chloride in serum rose to 100.3 mEq. per liter and that of sodium to 128, while potassium fell to 7.0 and carbon dioxide content by 2.8 mEq. per liter. Hiccoughs continued for 12 hours longer. He then received an additional 85 mEq. of sodium chloride by mouth. Within 36 hours his strength and sense of well being had been restored and the pulse and blood pressure had returned to their original values. However, without administration of salt, the concentration of sodium and chloride in serum fell about 3 mEq. per liter. The non-protein nitrogen did not fall, but serum potassium decreased further to 6.6 mEq. per liter on May 15th.

In the three day period prior to paracentesis, there had been retention of approximately 2.8 liters of water in excess of sodium (Table I, Figure 2) with an associated decline of 5 mEq. per liter in the concentration of sodium and chloride in serum. The calculated additional retention of water without salt during the 60 hours after paracentesis approximated only 800 ml. but the serum sodium declined by 5 and the chloride by 7 mEq. per liter during this period. During the 32 hour period after the administration of hypertonic saline, the increase in total body water as indicated by weight gain of 0.4 kilogram was small, but the increase in extracellular volume calculated from change in concentration of chloride in serum and chloride balance (17) was 2 liters. On May 13th, the patient began to leak fluid from the paracentesis puncture site and balance studies were discontinued.

Subsequently, a diet containing liberal amounts of sodium chloride was given and there were no further manifestations of salt deficiency. During the next two months there was slight clinical improvement and the abnormalities in tests of hepatic function became less marked. Additional paracenteses were required at monthly intervals. Some nitrogen retention persisted and endogenous creatinine clearance on June 22nd was only 29 ml. per minute. The gastric ulcer failed to heal and a second crater subsequently appeared. The patient died on August 4, 1950, following repeated episodes of gastro-intestinal bleeding. Findings at post mortem examination included portal cirrhosis of the liver with esophageal

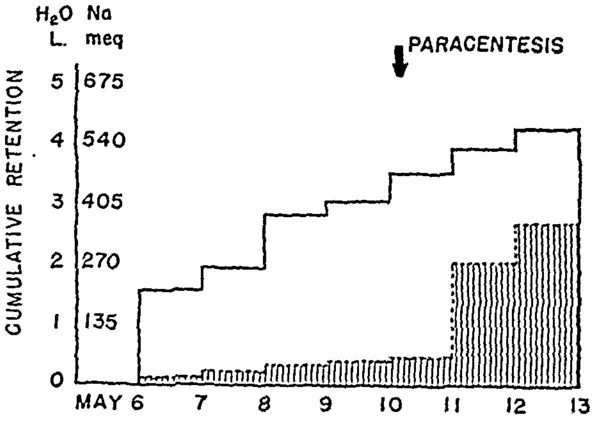

FIG. 2. CASE II. CUMULATIVE RETENTION OF TOTAL BODY WATER AND SODIUM (represented as in Figure 1)

Paracentesis performed late afternoon of May 9th.

varices; two benign gastric ulcers; and benign arteriolar nephrosclerosis of moderate severity.

## DISCUSSION

Manifestations observed in the first patient included hyponatremia, apathy, weakness, confusion, anorexia, nausea, vomiting, muscle cramps, hypotension, narrow pulse pressure, tachycardia, hemo-concentration and elevation of the serum non-protein nitrogen. Following the administration of hypertonic saline these disappeared. Furthermore, the syndrome failed to develop when isotonic saline was given after the second paracentesis. The second patient's symptoms were less severe but equally characteristic. The sensorium remained clear although depression and apprehension were noted. He became nauseated and had hiccoughs but did not vomit. Circulatory impairment, as reflected by hypotension and tachycardia, was unequivocal. Hyponatremia and hypochloremia were present. Nitrogen retention, present on admission, became more marked after paracentesis. The administration of sodium chloride produced marked improvement although the concentration of non-protein nitrogen and creatinine in the serum remained elevated.

In both patients, the urine volume remained small throughout, averaging about 600 ml. daily in Case I and approximately 750 ml. daily in Case II. Both patients retained water even though sodium was not given, so that hyponatremia necessarily developed. In Case I, although physical signs indicated rapid reaccumulation of ascitic fluid during the first 24 hours after the paracentesis of December 15th, the calculated increase in total extracellular fluid volume during this first day was negligible (0.13 liters) and the hematocrit rose but slightly, suggesting that losses of plasma water and electrolyte into the peritoneal cavity were being replaced at the expense of transfers from the interstitial extracellular compartment.

In the second patient, water was being retained in excess of sodium prior to paracentesis. Nevertheless, symptoms did not develop until about 60 hours after paracentesis. Although weight did not increase at an accelerated rate after paracentesis, the concentrations of sodium and chloride in serum underwent a further abrupt fall. The mechanism of this change is not clear, but requires either a shift of sodium chloride into cells or, due to alteration of intracellular osmolarity, a *movement of water out of cells.*

Both patients developed symptoms concomitant with the decline in concentration of sodium in serum during the days following paracentesis. This decline occurred despite the presence of an abnormally large total volume of extracellular fluid with an excessive total body content of sodium. However, with segregation in the peritoneal cavity of a considerable portion of the total volume of extracellular fluid (ascitic fluid plus interstitial fluid plus plasma), it appears likely that the extraperitoneal extracellular fluid volume (interstitial fluid plus plasma) was actually reduced. Certainly the plasma volume, as judged by the rising hematocrit in Case I, was diminished. Mankin and Lowell (18) observed that patients with cirrhosis characteristically reaccumulate ascites most rapidly during the first three days after paracentesis. In one of their patients ascitic fluid volume expanded by 2 liters during the first 24 hours after tapping, while body weight increased by only 1 kilogram. At least a liter of extraperitoneal extracellular fluid must therefore have been transferred into the peritoneal cavity. That transport of extraperitoneal extracellular fluid into the peritoneum frequently occurs after paracentesis is further supported by the clinical observation that when subcutaneous edema is present, it often decreases during this period, although body weight may not fall (19).

Several observations suggest that contraction of the extraperitoneal extracellular fluid volume (which, apart from any edema, might be called the "effective" extracellular volume) is an important feature of the syndrome. The second patient (W. F.) underwent two subsequent paracenteses after he had developed moderate dependent edema. No untoward manifestations appeared despite the fact that no extra salt was given and reaccumulation of ascites was initially rapid. The edema decreased without weight loss during the days following each paracentesis. This has been observed in other patients with ascites whose edema disappeared after paracentesis while their weight remained stationary and ascites increased. Presumably the edema fluid mobilized served to maintain the effective volume of extracellular fluid since symptoms did not appear despite moderate hyponatremia which was present before and after paracentesis and which showed no significant change after paracentesis. Others who have observed the occurrence of weakness and circulatory impairment after paracentesis state that they have encountered these symptoms only in patients with little or no peripheral edema who reaccumulate ascites rapidly (20, 21).

That hemoconcentration of some degree frequently follows paracentesis in patients with hepatic cirrhosis is evidenced by the observations of Gabuzda and his associates (19). A patient has been observed by us who developed severe hemoconcentration and became comatose on two occasions within 48 hours after paracentesis, and on each occasion was relieved by the intravenous infusion of salt poor human albumin. In the first case reported here, W. T., it seems probable that the administration of albumin might well have not only corrected the hemoconcentration but also the extreme thirst which presumably resulted from the contracted blood volume (22) and which directly led to the large water ingestion and consequent hyponatremia. Certainly the frequent occurrence of moderate hyponatremia in patients with decompensated cirrhosis without symptoms of salt deficiency (7, 8) suggests that hypotonicity alone, although hardly beneficial, does not produce the clinical syndrome described. It thus seems probable that a decrease in effective extracellular volume is a necessary event in its pathogenesis, and that the administration of hypertonic saline serves to correct both the hypotonicity and the diminished plasma volume.

Although the various manifestations encountered in the patients described above were similar to those encountered with diverse causes of salt deficit, the loss of water and electrolytes was into the peritoneal cavity rather than externally. The retention of ingested water without salt may have represented an attempt to restore the decreased effective extracellular volume or the diminished plasma volume at the expense of electrolyte concentration. If isotonic saline or edema fluid was available in sufficient quantity to provide for the obligatory reaccumulation of ascites, the syndrome did not occur. Once it had developed, however, hypertonic saline was necessary to restore electrolyte concentration and effective extracellular volume.

Rigid restriction of the intake of sodium has been widely practiced with a large measure of success in the management of patients with hepatic cirrhosis and ascites (7, 23–26). The infrequency of the development of the low-salt syndrome in patients so treated has been emphasized (24, 27). Although chronic hyponatremia in hepatic cirrhosis is common, it appears to be related to the severity of decompensation (7, 8) and does not necessarily depend upon rigid salt restriction. However, a tendency for serum sodium to fall in patients with hepatic cirrhosis treated by means of a diet poor in sodium has been reported by Farnsworth and Krakusin (28). Similar observations have been made by Eisenmenger and associates (8, 24), who described symptoms suggesting sodium deficit in two of 13 cases treated. However, in one patient the symptoms were very mild and in the second their relationship to sodium restriction was unclear. Symptomatic hypochloremia in hepatic cirrhosis was probably first described by Porges in 1932 (29). Mach and associates (30) recognized hypochloremia following repeated paracenteses, but observed no associated symptoms. Gabuzda and colleagues (19) have noted moderate hyponatremia to be a frequent transitory event after paracentesis, an observation confirmed by others (8).

It seems probable that if the syndrome described above occurred frequently in severe form, it would no doubt have been recognized more often in the past. The observations presented do not detract from the therapeutic value of salt restriction in cirrhosis, nor do they indicate that the large amounts of salt administered in this study will often be required. The available information suggests that the abnormality rarely becomes severe enough to produce symptoms. Moreover, these often may be mild and disappear without therapy specifically directed at their correction.

## SUMMARY

1. The development of symptomatic hyponatremia following abdominal paracentesis in two patients with cirrhosis of the liver has been described.

2. The manifestations were corrected by administration of sodium chloride in hypertonic solution.

3. Development of the syndrome was apparently prevented in one patient when his fluid intake after

paracentesis was limited to isotonic saline. In the other patient, when subcutaneous edema was present on a later occasion, its mobilization after paracentesis appeared to prevent the symptoms.

4. It is suggested that although total extracellular fluid volume was large, the extraperitoneal (effective) extracellular fluid volume was actually decreased in both patients when the syndrome developed.

## ACKNOWLEDGMENTS

The technical assistance of Miss Elsie C. Rossmeisl, Mrs. Eleanor G. Harkins, and Miss Helen G. Lichwell is gratefully acknowledged.

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# STUDIES OF RENAL OXYGEN CONSUMPTION IN MAN. I. THE EFFECT OF TUBULAR LOADING (PAH), WATER DIURESIS AND OSMOTIC (MANNITOL) DIURESIS[1]

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(Submitted for publication January 16, 1951; accepted April 23, 1951)

## INTRODUCTION

The energy demands of increased functional activity of the body as a whole are met by increased oxygen consumption with a high degree of thermodynamic efficiency amounting to 25 or 30 per cent (1). In the case of cardiac or skeletal muscle or secreting glands this relationship seems straightforward, but it is less obvious with brain or kidney. In the case of kidney, thermodynamic calculations by other workers (2, 3) indicate that the theoretical oxygen equivalent of the work necessary to produce maximal osmolar gradients between plasma and bladder urine is small relative to the resting metabolism of the organ. Experimental testing of this relationship by direct measurements in animals has resulted in contradictory reports (4–8). Since failure of experimental unanimity may have been due, in part, to differences in species, preparation or anesthesia, and since changes in renal oxygen consumption may be related to many factors other than mere overall osmotic gradients, it seemed advisable to make these measurements in unanesthetized man. Aside from their theoretical interest, such data might help in understanding the concepts of "renal work" and "resting the kidney" on the clinical level where they are stressed in all discussions of therapy but do not have an unequivocal background on the basis of either theory or experiment.

## METHODS

### *General*

Measurements of glomerular filtration rate, renal blood flow and renal oxygen consumption were made before and during test procedures in 15 adult male subjects between the ages of 17 and 58 years of age none of whom had evidence of renal disease. Clearances were performed in general according to the techniques described by Goldring and Chasis (9), and values were corrected to 1.73 square meters surface area. Constant infusions of test solutions, mannitol and para-aminohippurate (PAH), were given intravenously by means of a motor driven worm screw pushing on the barrel of a 50 cc. syringe according to the technique of Earle and Berliner (10). PAH and mannitol were mixed in the same solution; this procedure, however, causes no reduction in PAH extraction and clearance such as has been described with mixtures of PAH and glucose where presumably a coupling reaction occurs in which the p-amino group of the PAH is covered (11). Urine was collected by catheter with bladder washings. Blood samples were taken simultaneously from the femoral artery by means of an indwelling needle and from the right renal vein by means of a catheter. Before sampling, 5 cc. of blood were withdrawn from the artery and 10 cc. from the renal vein to clear the systems. These quantities were demonstrated to be adequate by testing *in vitro* with dye solutions. After sampling, this blood was reinjected. Blood to be analysed for excretory substances (mannitol, PAH and creatinine) was taken into dry syringes and transferred to tubes containing powdered heparin. Blood for gas analysis was taken anaerobically into 10 cc. syringes wet with liquid heparin. One cc. of mercury was introduced, the syringes were capped and kept in ice until analysed. This interval was sometimes as long as six hours in cases where a great many specimens were taken, but if the blood is adequately iced no measurable deterioration of oxygen content occurs in this time. Since analysis of each arterial sample was immediately followed by analysis of its paired venous sample, any undetected deterioration would tend to be minimized in the arterio-venous difference.

### *Test procedures*

After control observations varying from about 30 to 60 minutes' duration and consisting of two or three urine collection periods and one or two arterio-venous oxygen differences, one of the following experimental procedures, designed to change renal work, was instituted.

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[1] Presented to the American Physiological Society, Detroit, Mich., April 21, 1949.

[2] This work was done during the tenure of a National Institutes of Health Post Doctorate Fellowship.

[3] Harrison Fellow in Surgical Research.

[4] This work was supported by the Department of Medicine, The Harrison Department of Surgical Research and C. Mahlon Kline Fund for developments in the Department of Medicine.

*PAH saturation:* In five subjects the rate of excretion of PAH by the tubules was increased from values of 11–16 mg./min. to maximal rates of 71–86 mg./min. by increasing the rate of PAH infusion after an additional priming dose, thus raising the PAH plasma concentration from low levels of around 2 mg./100 cc. to higher levels of around 30 mg./100 cc.

*Water diuresis:* In five subjects tap water was given by mouth in varying amounts, depending on the subject's capacity, and measurements made after water diuresis had been established.

*Osmotic diuresis:* In five subjects mannitol was injected intravenously as a priming dose of 12.5 to 50 grams followed by a constant infusion of 194 to 262 mg./min.

### *Calculation of renal functions*

Renal blood flow (RBF) was determined by application of the Fick principle using PAH as a test substance, according to the equation $\text{RBF} = \frac{\text{UV}}{\text{A-R}} \times \frac{1}{\text{1-Hct}}$, where V equals the rate of urine formation in cc./min.; U, A and R equal the concentration of PAH in mg./cc. in urine, arterial plasma and renal venous plasma respectively; and Hct. is the arterial hematocrit. Determined values for A-R from simultaneously drawn samples were plotted against time on semilogarithmic paper and the interpolated value at the mid-point of each urine collection period was used to calculate RBF. Glomerular filtration rate (GFR) was measured as endogenous creatinine clearance (Ccr) in all cases and as mannitol clearance (Cm) in some. Total renal oxygen consumption ($\text{TQ}_{\text{O}_2}$) was calculated as the product of RBF and renal arterio-venous oxygen difference $(\text{A-R})_{\text{O}_2}$. Data have been expressed as cc./100 grams/min. ($\text{Q}_{\text{O}_2}$) on the assumption that 300 grams of renal tissue are equivalent to 1.73 square meters surface area, *i.e.*, $\text{Q}_{\text{O}_2} = \text{TQ}_{\text{O}_2}/3$. In each case, control values of RBF and $(\text{A-R})_{\text{O}_2}$ were averaged and these averages multiplied together to calculate $\text{TQ}_{\text{O}_2}$. Experimental values were treated in the same manner. To avoid the possibility of error in $(\text{A-R})_{\text{O}_2}$ estimations, arising from changes in hemoglobin concentration in passage through the kidney or by unequal dilution in the sampling syringes by liquid heparin or traces of saline remaining in the venous catheter, values for renal venous oxygen content have been corrected to the same hemoglobin content as the simultaneously drawn specimen from the femoral artery according to the following formula. $\text{R}_{\text{O}_2\text{C}} = \text{R}_{\text{O}_2\text{D}} \times \text{AHb/RHb}$, when $\text{R}_{\text{O}_2\text{C}}$ is the corrected, and $\text{R}_{\text{O}_2\text{D}}$ the determined value for oxygen content of renal venous blood in vols. per cent, and AHb/RHb is the ratio of hemoglobin concentration in the femoral artery to that in the renal vein. This value, AHb/RHb, averaged 0.997 in 47 determinations with a standard deviation of 0.027 and a standard error of the mean of 0.004. The range, however, was from 0.913 to 1.050, and while these corrections are small compared to the total oxygen content of either arterial or renal venous blood, they may constitute a sizeable fraction of the relatively small $(\text{A-R})_{\text{O}_2}$. In 11 instances the correction was zero but in 10 it was greater than 0.50 vol. per cent. The mean of the differences $\text{R}_{\text{O}_2\text{C}}\text{-R}_{\text{O}_2\text{D}}$ was not statistically significantly different from zero but this merely indicates that AHb tended to exceed RHb as frequently as RHb exceeded AHb. These changes are probably not due to errors in the hemoglobin method, since we have found in 42 sets of triplicate determinations (assuming 1 gram of hemoglobin to be equivalent to 1.34 cc. of oxygen) that the maximum change in $(\text{A-R})_{\text{O}_2}$ resulting from error in AHb/RHb should have been less than 0.2 vol. per cent in 95 per cent of the cases. On the basis of subsequent work, we are inclined to believe that one factor in this difference was unequal dilution of the arterial and renal venous blood by the liquid heparin in the sampling syringes. Similar results of about the same magnitude have been reported by other workers in the dog (1, 12, 13) although smaller differences have been found in man (14, 15).

### *Chemical determinations*

Blood oxygen content was determined in duplicate on 1 cc. samples by the manometric method of Van Slyke and Neill for simultaneous determinations of carbon dioxide and oxygen (16). In all determinations gas was thus liberated by means of a combined reagent containing lactic acid. Under these conditions, as has been demonstrated in this laboratory (17) and recently confirmed (18), the inaccuracies of oxygen determinations resulting from incomplete hemolysis of red cells by saponin in neutral solution do not occur (19). Duplicate determinations agreed within 0.05 vol. per cent for the most part and 0.10 vol. per cent in all cases.

PAH was determined as "total" PAH by acid hydrolysis of the conjugated material according to a modification of the method of Smith and his associates (20) in 11 cases and by the method of Newman and his associates (21) in the last four.

Mannitol was determined by a modification of Smith's method (22); endogenous creatinine was determined by the method outlined by Brod and Sirota (23); oxyhemoglobin determinations were done on the Evelyn photoelectric colorimeter (24) and hematocrits were determined according to the method of Wintrobe (25) with no correction for trapped plasma.

### *Statistical methods*

To evaluate results, the standard error of measurement, $\sigma m$, for each specific function has been calculated from the variability of multiple control determinations in each individual according to the formula $\sigma m = \sqrt{\frac{\epsilon d^2}{n'}}$, where d represents the deviation of each observation from the mean value for that subject and n′ represents degrees of freedom calculated as the total number of observations minus the total number of subjects. The standard error of measurement of the mean of multiple determinations in a single subject equals $\frac{\sigma m}{\sqrt{n}}$ where n is the number of observations making up the mean. In the case of $\text{Q}_{\text{O}_2}$, the product of $(\text{A-R})_{\text{O}_2}$ and RBF, the standard error of measurement has been calculated from the formula:

$$\left(\frac{\sigma m T Q_{O_2}}{\overline{TQ_{O_2}}}\right)^2 = \left(\frac{\sigma m (A\text{-}R)_{O_2}}{\overline{(A\text{-}R)_{O_2}}}\right)^2 + \left(\frac{\sigma m RBF}{\overline{RBF}}\right)^2$$

where $\overline{Q_{O_2}}$, $\overline{(A\text{-}R)_{O_2}}$ and $\overline{RBF}$ refer to the respective means. Dividing by three expresses $\sigma m Q_{O_2}$ in cc./100 grams/min.

Standard errors of measurement thus calculated for the various specific renal functions are shown in Table I. The standard error of measurement of the difference, $\sigma md$, of each experimental value from its own control value was calculated according to the formula $\sigma md$ equals $\sqrt{\sigma m^2 c + \sigma m^2 e}$ where $\sigma mc$ and $\sigma me$ are the standard errors of measurement of the control and experimental values respectively. Each difference was then divided by its standard error of measurement and the resulting "t" value evaluated for significance at the 5 per cent level.

The significance of the means of the differences between control and experimental observations for all subjects taken together within each of the three test groups was evaluated by the usual "t" test.

## RESULTS[5]

The data are given in Table II. None of the test procedures used resulted in consistent measurable changes in either $(A\text{-}R)_{O_2}$ or $Q_{O_2}$ and in only a single instance, $Q_{O_2}$ in patient 6, was the change significantly greater than might be expected on the basis of the error of the method. If one disregards patients 1, 4, and 6 there was no change in $(A\text{-}R)_{O_2}$ greater than 0.4 vol. per cent or in $Q_{O_2}$ greater than 1.4 cc./100 grams/min. Thus on a statistical basis no t value would have been greater than 0.7. Likewise the mean differences are not statistically significant. Glomerular filtration rate as estimated by endogenous creatinine increased significantly with water diuresis. At the onset of water diuresis, usually, for a single urine collection period, some of the subjects showed a transient increase in RBF (persistent in patient 8) which may have been due to hyperemia but may also possibly have been due to washout of PAH contained in the urinary dead space as the rate of diuresis increased. Such periods were not included in experimental averages and $(A\text{-}R)_{O_2}$ measurements were not made at times when it was anticipated that this change would occur, so as to avoid any possible effect on $Q_{O_2}$ of hemodynamic changes *per se*, real or apparent, in contradistinction to effect on $Q_{O_2}$ of changes in renal tubular functional activity.

TABLE I

*Statistical basis for evaluation*

| Specific renal function | Standard error of measurement when *n* equals | | |
|---|---|---|---|
| | *1* | *2* | *3* |
| GFR cc./min. | | | |
| Mannitol clearance | 8.4 | 6.0 | 4.9 |
| Creatinine clearance | 7.5 | 5.3 | 4.3 |
| RBF cc./min. | 168 | 118 | 97 |
| $(A\text{-}R)_{O_2}$ vols. per cent | .37 | .26 | .21 |
| $Q_{O_2}$ cc./100 grams/min. | | | |
| Single $(A\text{-}R)_{O_2}$ × mean of n RBF | 1.90 | 1.82 | 1.79 |
| Mean of 2 $(A\text{-}R)_{O_2}$ × mean of n RBF | 1.45 | 1.34 | 1.30 |

The mean renal arterio-venous differences of the 15 subjects calculated from control observations only was 1.43 vols. per cent with a standard deviation of 0.39 and a standard error of the mean of 0.10. The mean renal oxygen consumption was 6.24 cc./100 grams/min. with a standard deviation of 2.07 and standard error of the mean of 0.54. These values are in good agreement with those of other workers for the metabolism of the normal kidney (14, 15, 27).

## DISCUSSION

These data indicate that, if changes in renal oxygen consumption occurred under the conditions of these experiments, such changes were masked by the error of measurement. While these measurements will detect only relatively large changes, they would be quite adequate to detect such changes as may occur in the body as a whole during exercise where oxygen consumption may increase 16 fold (28). Failure to obtain such a response means either that the stimuli were inadequate or that the kidney uses most of its oxygen for something other than the changes in work load we induced. Since the kidney is never entirely at rest, it may well be that the differences between control and experimental conditions were really quite small with respect to the total energy required in the complex process of converting glomerular filtrate into urine and maintaining the functional state of the renal cells. A relationship between urinary function and renal oxygen consumption is suggested by the data of Cargill and Hickam

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[5] Following the presentation of these data in abstract form, Bucht, Werkö, and Josephson have reported similar results associated with tubular saturation with PAH (26).

TABLE II

*Effect on renal oxygen consumption of some changes in functional activity*

| Patient | Tubular* excretion PAH *mg./min.* | | Rate of diuresis *cc./min.* | | Urine specific gravity | | U/P ratio creatinine | | Filtration rate (GFR)† *cc./min.* | | Renal blood flow (RBF) *cc./min.* | | Renal A-V $O_2$ difference $[(A-R)_{O_2}]$‡ *vols. per cent* | | Renal $O_2$ consumption ($Q_{O_2}$) *cc./100 grams/min.* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C§ | E§ | C | E | C | E | C | E | C | E | C | E | C | E | C | E |
| I. PAH saturation | | | | | | | | | | | | | | | | |
| 1 | 16 | 86 | | | | | | | —‖ | 92¶ | —‖ | 1270¶ | 0.9¶ | 2.0¶ | 3.8¶ | 8.5¶ |
| 2 | 17 | 79 | | | | | | | 133 | 127 | 1900 | 1690 | 1.1 | 1.0¶ | 7.0 | 5.6¶ |
| 3 | 13 | 71 | | | | | | | 92 | 108 | 2000 | 1845¶ | 0.6 | 0.8¶ | 4.0 | 4.9¶ |
| 4 | 13 | 79 | | | | | | | 95 | 81 | 1310 | 1160 | 1.1 | 0.4¶ | 4.8 | 1.5¶ |
| 5 | 11 | 80 | | | | | | | 83 | 91 | 764¶ | 819 | 1.5 | 1.8¶ | 3.8 | 4.9¶ |
| Mean | 14 | 79 | | | | | | | 101 | 102 | 1490 | 1380 | 1.04 | 1.20 | 4.68 | 5.08 |
| II. Water diuresis | | | | | | | | | | | | | | | | |
| 6 | | | 0.5 | 7.5 | | | 163 | 9 | 65 | 70 | 1840 | 1690 | 1.6 | 0.8¶ | 9.8 | 4.5¶** |
| 7 | | | 0.8 | 6.6 | | | 135 | 17 | 99 | 115** | 1350 | 1560 | 2.1¶ | 1.8¶ | 9.5¶ | 9.4¶ |
| 8 | | | 0.5 | 12.5 | 1.022 | 1.003 | 232 | 11 | 116 | 137** | 1540 | 1830** | 1.6 | 1.3¶ | 8.2 | 7.9¶ |
| 9 | | | 0.8 | 8.0 | 1.018 | 1.001 | 119 | 12 | 95 | 98 | 878 | 884 | 1.6 | 2.0 | 4.7 | 5.9 |
| 10 | | | 0.8 | 3.1 | 1.022 | 1.009 | 83 | 22 | 66 | 69 | 855 | 923 | 1.7 | 1.5¶ | 4.8 | 4.6¶ |
| Mean | | | 0.7 | 7.5 | | | 146 | 14 | 88 | 98** | 1093 | 1177 | 1.72 | 1.48 | 7.40 | 6.46 |
| III. Mannitol diuresis | | | | | | | | | | | | | | | | |
| 11 | | | 0.9 | 3.1 | | | 121 | 29 | 109 | 108 | 1400 | 1430 | 1.8¶ | 1.6¶ | 8.4¶ | 7.6¶ |
| 12 | | | 0.5 | 4.4 | | | 156 | 18 | 78 | 79 | 1060 | 1110 | 1.6 | 1.6 | 5.7 | 5.9 |
| 13 | | | 0.4 | 6.3 | | | 215 | 13 | 86 | 85 | 984 | 1120 | 1.8 | 1.9 | 5.9 | 7.1 |
| 14 | | | 0.7 | 7.8 | | | 237 | 22 | 166 | 172 | 2210 | 2550** | 1.2 | 1.1 | 8.8 | 9.4 |
| 15 | | | 0.6 | 4.6 | | | 170 | 22 | 102 | 101 | 935 | 915 | 1.4 | 1.6 | 4.4 | 4.9 |
| Mean | | | 0.6 | 5.2 | | | 180 | 21 | 108 | 109 | 1318 | 1425 | 1.56 | 1.56 | 6.64 | 6.98 |
| Grand mean | | | | | | | | | | | | mean<br>$\sigma$<br>$\sigma\bar{x}$ | 1.43<br>0.39<br>0.10 | | 6.24<br>2.07<br>0.54 | |

* Calculated by $Tm_{PAH}$ formula $T = UV - 0.83\ GFR \times P_{PAH}$, where T = tubular excretion of PAH, UV = urinary excretion of PAH, GFR = glomerular filtration rate, $P_{PAH}$ = plasma concentration of PAH.

† Mannitol clearance in I, endogenous creatinine clearance in II and III.

‡ Corrected to equal hemoglobin concentrations.

§ C = control, E = experimental determination.

‖ Values not obtained due to technical difficulties, experimental value of RBF used to calculate $Q_{O_2}$ since variability of $(A-R)_{O_2}$ is much greater than that of RBF.

¶ Single determination, all others average of 2 or 3.

** Statistically significant change from control value.

(15) who found a significant correlation between glomerular filtration rate and renal oxygen consumption. Their studies, however, included observations on patients with renal disease where, as the authors point out, metabolic properties may be altered. Hayman and Schmidt in 1928 (5) and Van Slyke and his associates in 1934 (8), on the basis of data in animals similar to these in humans, postulated that the bulk of energy resulting from renal oxygen consumption is expended for something other than external work.

Rapoport and his colleagues have determined that calculated renal osmotic work can be increased by solute loading up to a maximum value of about 4.0 calories/min./1.73 square meters (3). On this basis, since 1 cc. of oxygen is approximately equivalent to 5 calories (only minor errors would be introduced by varying R. Q.), a change from resting state to one of maximal osmotic work should cause an increase in $Q_{O_2}$ of roughly 0.3 cc./100 grams/min., or only 5 per cent of our mean value for oxygen consumption of the human kidney. As these authors have pointed out (29), one may question the physiologic significance of such thermodynamic calculations since they consider only the overall osmolar gradient between plasma and bladder urine, and processes such as glucose reabsorption, which might require considerable energy expenditure, are not represented as net work, since they are balanced by water reabsorption with no osmolar change in tubular urine.

Nevertheless, the fact that *in vitro* determinations of renal oxygen consumption where no urine is being formed (30, 31), when recalculated into the same units as our data, give results of comparable magnitude also supports the concept that most of the oxygen used by the kidney is not used for external work.

Thus the metabolic state of the kidney might be regarded as one of dynamic equilibrium in an open-ended system as recently discussed by von Bertalanffy (32). In such a system the bulk of energy is expended to maintain the steady state which enables work to be done, and external functional processes, such as urine formation, could be accomplished with little added energy beyond the basic needs of the organ.

Applied to the clinical level, if it be true that the great bulk of renal metabolism goes on more or less independently of osmotic work being done, such a concept might suggest that whatever may be the value of special diets in the treatment of renal disease, such value is not dependent on "resting the kidney" by decreasing solute load.

## SUMMARY

1. By means of clearance techniques with renal vein catheterization renal oxygen consumption has been measured in unanesthetized man before and during (*a*) saturation of the tubular transport mechanism for PAH, (*b*) water diuresis, and (*c*) osmotic diuresis induced by mannitol.

2. The mean renal oxygen consumption in 15 subjects was 6.24 cc./100 grams/min. with a standard deviation of 2.07 and a standard error of the mean of 0.54.

3. The mean renal arterio-venous oxygen difference was 1.43 vols. per cent with a standard deviation of 0.39 and a standard error of the mean of 0.10.

4. These values are in reasonably close agreement with *in vitro* work.

5. There was no statistically significant change in renal oxygen consumption between the control and test measurements.

6. These data suggest that the bulk of oxygen consumed by the kidney is used for purposes not measurable by external work.

7. The data suggest that any beneficial results of special diets in renal disease do not result from resting the kidney by decreasing the oxygen requirement as a result of diminution of external work.

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# THE EFFECTS OF AN INCREASE IN PLASMA VOLUME ON THE METABOLISM AND EXCRETION OF WATER AND ELECTROLYTES BY NORMAL SUBJECTS[1, 2]

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(Submitted for publication January 22, 1951; accepted April 23, 1951)

## INTRODUCTION

Since the volume of body water is maintained within reasonable limits and the kidneys play a dominant role in this regulation, it is implicit that the existence of an abnormal volume is somehow transmitted to the kidney. In turn, some renal mechanism must be stimulated to alter the volume and composition of the urine in a manner that will tend to correct the abnormality. There may be *volume receptors* as such or the responsive mechanism may be stimulated by some *function* of an abnormal volume. The one compartment of body water whose volume is most readily altered by changes in the others and whose constancy is most important for a host of physiologic processes is the circulating plasma. It is, therefore, important to learn what alterations in renal function are associated with changes in plasma volume and in what manner these responses may be modified.

The volume of the plasma can be increased without alterations in the volumes of the other compartments of fluid by the infusion of a solution identical with plasma both with respect to filtrable constituents and colloid osmotic pressure. Solutions of gelatin, acacia, plasma, and albumin have been used therapeutically with success for this purpose. The infusion of a 25% or 10% solution of albumin will expand the volume of the plasma at the expense of the interstitial fluid because such a solution is hyperoncotic with respect to the plasma of the recipient. Thus, by varying the concentration of the solution of albumin infused, an increase in plasma volume with or without a change in the interstitial fluid volume can be achieved.

Many investigators have studied this problem in the experimental animal and in man with conflicting results (1–5). This investigation is an attempt to study the alterations in the metabolism and excretion of electrolytes and water promoted by expansion of the plasma volume with hyper- and isooncotic solutions of purified salt-poor human albumin.

## EXPERIMENTAL PROCEDURE AND METHODS

The experimental subjects in this study were two healthy physicians (J. O. and L. G. W.), one patient with well-controlled Addison's disease (R.N.), and four patients (B., B. B., S., and A.) with minor diseases unrelated to the cardiovascular-renal systems. Food and water were restricted for a period of 10 to 14 hours prior to each experiment except in L. G. W. 12–11–48 when fluids as such were restricted for 48 hours. Each experiment was conducted with the subject in the recumbent position, either on a couch or in bed, and was of six hours' duration except as indicated. The experiments were divided into three major periods: pre-infusion control, infusion, and post-infusion. All urines were voided specimens. Samples of blood were collected under oil at the end of each period.

All of the experiments except those noted in the tables as "evening studies" (L. G. W. 10–25–48 and J. O. 2–1–49) were started at about 8 A.M. The "evening studies" were started at 6 P.M. after abstention from food and water for 10 hours.

No water was ingested during any of the studies where 4%, 5%, 6%, and 10% solutions of albumin were infused, and in the experiments where 2,500 cc. of saline were infused prior to the administration of 25% salt-poor albumin. In all the other experiments in which a 25% solution of albumin was administered (except L. G. W. 12–11–48) water was ingested at the rate of 100 cc. an hour.

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[1] Serum albumin used in this study was prepared by the American Red Cross from blood of volunteer donors. The conclusions are those of the authors and do not necessarily reflect the policy of the American Red Cross.

[2] Aided by an institutional grant from the National Heart Institute, U. S. Public Health Service, and Contract V1001 M-1450 of the Veterans Administration.

[3] Work done in part during the tenure of a Post-doctorate Fellowship, U. S. Public Health Service.

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The control studies were designed to mimic the experiments under consideration in all details except for the protein in the infusion fluid. The concentration of sodium and chloride in 25% salt-poor human albumin is 157 and 20 mEq./L respectively, and the water content is approximately 80%. The control infusion for 400 cc. of 25% salt-poor human albumin was, therefore, 325 cc. of an aqueous solution containing the same amount of sodium and chloride using appropriate amounts of NaCl and $NaHCO_3$. The control infusion for 1,000 cc. of 10% albumin in saline was 1,000 cc. of normal saline. An equal volume of 5% glucose was used to control the experiment with 4% albumin in 5% glucose, and an equal volume of normal saline was used to control the studies with 4%, 5%, and 6% albumin in normal saline. Water was ingested at the rate of 100 cc. an hour when this had prevailed in the experiment for which the control study was performed. In the other control studies no water was ingested.

The chemical methods and calculations have been described in previous publications from this department (6–9). In the calculations the value for the initial volume of extracellular fluid (chloride space) was assumed to be 20% of the body weight. The initial plasma volume was assumed to be 5% of the body weight. The value for total milli-osmols in the urine was calculated from the equation: $2(Na + K) + urea$. The percentage change in plasma volume, $PV_2/PV_1$, was calculated from the formula: $\frac{Hgb_1}{Hgb_2} \times \frac{1 - Hkt_2}{1 - Hkt_1} \times 100$.

In all the experiments, except L. G. W. 10–18 and 10–25–48 and J. O. 10–20–48, the concentration of creatinine in the serum was determined and the clearance of creatinine was calculated from the formula $\frac{UV}{S}$. In the three experiments, cited above, only the excretion of creatinine was determined and the concentration of creatinine in the serum was assumed to be constant at 1 mgm.% for the purpose of calculating the clearance of creatinine. Urea clearances were calculated from the formula, $\frac{UV}{B}$, regardless of the rate of urine flow.

## RESULTS

The infusion of 50 or 100 gm. of a hyperoncotic solution (25% or 10%) of albumin was invariably associated with an increase in the concentration and total circulating quantity of serum albumin and an enlarged plasma volume (Table I). There was a reciprocal fall in the concentration of serum globulin with a small and inconstant increase in total circulating globulin. Since there were no significant changes in the volume of the extracellular fluid, the increase in plasma volume was associated with a reciprocal fall in the volume of the interstitial fluid.

The calculated increase in total circulating albumin agreed quite closely with the quantity injected in six of nine studies. The discrepancies probably represent the compounding of errors inherent in the determinations involved. The percentage increase in plasma volume varied from 20% to 51%, but was not necessarily related to the quantity of albumin injected. The increase in plasma volume per gram of albumin infused was greater when the protein was administered in less concentrated solutions (Tables I and II). The increase in plasma volume expressed as cubic centimeters per gram of albumin infused varied from 7.95 to 18.2 cc. with an average of 13.2 cc. when hyperoncotic solutions were infused, and from 10.2 to 28.8 cc. with an average of 18.9 cc. when hypo- or isooncotic solutions were administered. Infusions of hyperoncotic solutions of albumin after prior expansion of the extracellular space with 2½ liters of normal saline were associated with an increase in plasma volume of 11.6 cc. per gm. There were no consistent significant changes in the size of the red cells as indicated by the values for Hgb/Hkt.

Infusions of hyperoncotic (25% or 10%) solutions of albumin in the experiments conducted in the morning (Table III) were associated with no consistent change in the clearance of endogenous creatinine. The infusions were associated with no change or a fall in the rates of excretion of sodium and chloride when compared to the pre-infusion control periods. There were *no* instances where the rate of excretion of either of these two ions was *augmented.* In contrast, in the control study for L. G. W. 10–18–48, where the infusion had the same volume of water and content of sodium and chloride as the 25% solution of albumin, the rates of excretion of sodium and chloride were distinctly *increased.* This same difference in the response to a non-colloid solution of equal volume and salt content as compared to an infusion of a 10% solution of albumin in normal saline is evident by inspecting the results in experiment J. O. 1–8–49 and its control (Table III).

It is more difficult to analyze the response in the rate of excretion of water associated with the administration of a 25% solution of albumin, since in all but one of these studies water was ingested at a rate of 100 cc./hr., which would be expected to increase the rate of flow of urine. In the experiment

TABLE I

*The effect of hyperoncotic solutions of albumin on the serum proteins and the volumes of the plasma and interstitial fluids*

| Study | Period | Duration | Intravenous albumin | Conc. in serum | | | Hkt | Hgb | $\frac{Hgb}{Hkt}$ | $\frac{PV_2}{PV_1}$ | Δ PV per gm. albumin | Δ Total circul. albumin | Δ Total circul. globulin | Δ IFV |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Total protein | Albumin | Globulin | | | | | | | | |
| | | *min.* | *gm.* | *gm. %* | *gm. %* | *gm. %* | *vol. %* | *gm. %* | | % | *cc.* | *gm.* | *gm.* | *liters* |
| L. G. W. | I | 118 | | 6.40 | 4.15 | 2.25 | 46.0 | 15.1 | 32.8 | 100 | | | | |
| 10-11-48 | II | 60* | 50 | 7.24 | 5.17 | 2.07 | 40.4 | 13.6 | 33.7 | 123 | 18.2 | 87.2 | 10. | −0.870 |
| 25% | III | 60 | | 7.21 | 5.06 | 2.15 | 38.6 | 13.8 | 35.8 | 124 | | | | −1.110 |
| | IV | 60 | | 7.21 | 4.90 | 2.31 | 40.1 | 13.6 | 33.9 | 123 | | | | −0.760 |
| L. G. W. | I | 120 | | 6.87 | 4.45 | 2.42 | 45.2 | 14.7 | 39.4 | 100 | | | | |
| 10-18-48 | II | 57* | 50 | | | | | | | | | | | |
| 25% | III | 78* | 50 | 7.91 | 5.94 | 1.97 | 42.0 | 13.0 | 37.9 | 120 | 7.95 | 106.5 | −2.2 | −0.475 |
| | IV | 120 | | 7.62 | 5.76 | 1.86 | 40.5 | 13.1 | 39.5 | 122 | | | | −0.790 |
| L. G. W. | I | 120 | | 7.07 | 4.52 | 2.55 | 42.0 | 13.8 | 32.9 | 100 | | | | |
| 10-25-48 | II | 59* | 50 | | | | | | | | | | | |
| Evening study | III | 61* | 50 | 8.20 | 6.10 | 2.10 | 37.0 | 12.0 | 32.5 | 125 | 10.0 | 126.5 | 3.2 | −0.890 |
| 25% | IV | 120 | | 7.70 | 5.82 | 1.88 | 38.7 | 12.6 | 32.6 | 115 | | | | −0.930 |
| L. G. W. | I | 120 | | 7.43 | 4.27 | 3.16 | 46.3 | 15.6 | 33.7 | 100 | | | | |
| 12-11-48 | II | 60* | 50 | | | | | | | | | | | |
| Dehydrated | III | 60* | 50 | 8.45 | 6.29 | 2.16 | 38.7 | 12.8 | 33.1 | 139 | 15.3 | 175 | −6.3 | −1.38 |
| 25% | IV | 120 | | 8.15 | 5.92 | 2.23 | 40.5 | 12.8 | 31.6 | 135 | | | | −1.19 |
| J. O. | I | 120 | | 6.86 | 4.59 | 2.26 | 41.9 | 13.8 | 32.9 | 100 | | | | |
| 10-15-48 | II | 60* | 50 | 7.06 | 5.15 | 1.91 | 37.6 | 12.1 | 32.2 | 122 | 15.0 | 59.6 | 2.5 | −0.885 |
| 25% | III | 60 | | | | | | | | | | | | |
| | IV | 60 | | 7.17 | 5.17 | 2.00 | 34.9 | 12.4 | 35.5 | 125 | | | | |
| J. O. | I | 120 | | 6.81 | 4.61 | 2.20 | 39.6 | 13.5 | 34.1 | 100 | | | | |
| 10-20-48 | II | 63* | 50 + | | | | | | | | | | | |
| 25% | III | 58* | 50 | 8.24 | 6.23 | 2.01 | 35.6 | 11.7 | 32.9 | 123 | 8.1 | 108 | 9.7 | −1.03 |
| | IV | 120 | | 8.17 | 6.06 | 2.11 | 36.7 | 12.3 | 33.5 | 115 | | | | −1.12 |
| J. O. | I | 120 | | 6.75 | 4.35 | 2.40 | 44.9 | 13.8 | 30.7 | 100 | | | | |
| 1-8-48 | II | 60* | 50 | | | | | | | | | | | |
| 10% | III | 60* | 50 | 7.46 | 5.34 | 2.12 | 35.7 | 11.4 | 31.8 | 142 | 15.7 | 121 | 22.7 | −1.04 |
| | IV | 120 | | 7.46 | 5.40 | 2.06 | 38.8 | 12.0 | 30.9 | 128 | | | | −0.52 |
| J. O. | I | 120 | | 7.58 | 4.77 | 2.81 | 41.8 | 13.2 | 31.6 | 100 | | | | |
| 2-1-49 | II | 60* | 50 | | | | | | | | | | | |
| Evening study | III | 60* | 50 | 7.97 | 5.89 | 2.08 | 35.6 | 10.9 | 30.6 | 134 | 12.2 | 111.8 | −1 | −1.110 |
| 25% | IV | 120 | | 7.82 | 5.95 | 1.87 | 38.0 | 11.8 | 31.1 | 119 | | | | −0.630 |
| R. N. | I | 120 | | 7.64 | 3.77 | 3.87 | 40.4 | 12.2 | 30.2 | 100 | | | | |
| 1-13-49 | II | 60* | 50 | | | | | | | | | | | |
| 25% | III | 60* | 50 | 8.08 | 4.95 | 3.13 | 30.7 | 9.4 | 30.4 | 151 | 16.6 | 121 | 28 | −1.91 |
| | IV | 120 | | 7.79 | 5.18 | 2.61 | 35.3 | 9.8 | 27.8 | 135 | | | | −1.32 |

* Period of infusion.

L. G. W. 10-18 and its control (Table III) the rates of excretion of water do not differ significantly. In the one study where a 25% solution of albumin was injected and no water was taken by mouth (L. G. W. 12-11-48) there was a decrease in the rate of excretion of water in the post-infusion period. Comparison of the rates of excretion of water in the experiments J. O. 1-8-49 and its control reveals that the hyperoncotic solution of albumin (10%) provoked a mild antidiuretic response. It should be noted, however, that this antidiuresis was associated with a decrease in the concentration of total milliosmols in the urine. These responses with respect to the rates of excretion of sodium and water associated with infusions of hyperoncotic solutions of albumin in normal subjects are in striking contrast to the responses in patients with the nephrotic syndrome and toxemias of pregnancy (10-13).

Two subjects (Table IV) were given an infusion of 100 cc. (50 gm.) of 25% albumin after the extracellular space had been expanded with

2,500 cc. of normal saline. These studies were controlled by the administration of 200 cc. of normal saline in lieu of the albumin solution after the initial expansion of the extracellular space. Despite prior expansion of the volume of extracellular fluid, the infusion of a solution of 25% albumin did not promote an increase in the rates of excretion of sodium or water. On the contrary, the albumin infusion clearly retarded the rate of excretion of sodium, chloride, potassium, and water in subject B. B.

Infusions of hyperoncotic solutions of albumin at night were associated with a somewhat different response than those observed during the morning (Table V). The rates of excretion of sodium and chloride in the control period are distinctly lower than they were in the comparable period in the morning. The infusion of 100 gm. of 25% albumin in both subjects and the control infusion were each followed by a slight increase in the rate of excretion of sodium and a decrease in the rates of excretion of both chloride and potassium. Examination of the rates of excretion of water in experiment J. O. 2–1–49 and its control reveals no significant difference.

The responses provoked by infusions of iso- and hypo-oncotic solutions of albumin are in striking contrast to the foregoing (Table VI). Infusions of 2,280 cc. of 4%, 5%, and 6% solutions of albumin in saline, and 1,825 cc. of a 4% solution of albumin in 5% glucose promote a striking increase in the rate of excretion of water. This di-

TABLE II

*The effect of hypo- and iso-oncotic solutions of albumin on the serum proteins and the volumes of the plasma and interstitial fluid*

| Study | Period | Duration | Intravenous albumin | Conc. in serum | | | Hkt | Hgb | $\frac{Hgb}{Hkt}$ | $\frac{PV_2}{PV_1}$ | Δ PV per gm. albumin | Δ Total circul. albumin | Δ Total circul. globulin | Δ IFV |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Total protein | Albumin | Globulin | | | | | | | | |
| | | *min.* | *gm.* | *gm. %* | *gm. %* | *gm. %* | *vol. %* | *gm. %* | | % | *cc.* | *gm.* | *gm.* | *liters* |
| L. G. W.<br>1–12–49<br>4% | I | 120 | | 6.93 | 3.78 | 3.15 | 45.5 | 14.4 | 31.7 | 100 | | | | |
| | II | 60* | 45.6 | | | | | | | | | | | |
| | III | 60* | 45.6 | 7.19 | 4.77 | 2.42 | 35.5 | 11.5 | 32.4 | 148 | 20.2 | 125.2 | 16.4 | −0.630 |
| | IV | 120 | | 7.37 | 4.29 | 3.08 | 39.0 | 13.0 | 33.3 | 124 | | | | −0.390 |
| L. G. W.<br>2–17–49<br>5% | I | 120 | | 6.87 | 4.36 | 2.51 | 45.6 | 14.7 | 32.3 | 100 | | | | |
| | II | 60* | 57 | | | | | | | | | | | |
| | III | 60* | 57 | 7.54 | 5.26 | 2.28 | 36.0 | 11.4 | 31.7 | 152 | 18.5 | 145.5 | 38.1 | −0.510 |
| | IV | 120 | | 7.15 | 5.09 | 2.06 | 36.5 | 11.8 | 32.3 | 145 | | | | −0.510 |
| L. G. W.<br>2–24–49<br>6% | I | 120 | | 7.26 | 4.86 | 2.40 | 44.5 | 14.8 | 33.3 | 100 | | | | |
| | II | 60* | 68.4 | | | | | | | | | | | |
| | III | 60* | 68.4 | 7.97 | 6.34 | 1.63 | 33.5 | 13.1 | 39.1 | 135 | 10.2 | 146.5 | −7.8 | +0.08 |
| | IV | 120 | | 7.66 | 5.84 | 1.82 | 30.5 | 9.8 | 32.2 | 189 | | | | −1.919 |
| J. O.<br>1–22–49<br>4% | I | 120 | | 7.17 | 4.25 | 2.92 | 41.3 | 13.3 | 32.2 | 100 | | | | |
| | II | 60* | 37.5 | 6.79 | | | | | | | | | | |
| | III | 60* | 37.5 | 7.38 | 4.83 | 2.55 | 35.0 | 11.3 | 31.8 | 131 | 15.5 | 77.6 | 15.7 | −0.460 |
| | IV | 120 | | 7.51 | 5.05 | 2.46 | 39.6 | 12.4 | 31.3 | 111 | | | | −0.110 |
| J. O.<br>2–10–49<br>Control for<br>J. O. 1–22–49 | I | 120 | | 7.35 | | | 41.2 | 13.1 | 38.8 | 100 | | | | |
| | II | 60* | | 6.58 | | | | | | | | | | |
| | III | 60* | | 6.57 | | | 39.6 | 12.2 | 37.9 | 110 | | | | +0.149 |
| | IV | 120 | | 7.39 | | | 41.1 | 13.3 | 39.4 | 99 | | | | +0.265 |
| S.<br>6–5–50<br>4% | I | 123 | | 6.68 | 3.37 | 3.31 | 41.1 | 12.3 | 29.9 | 100 | | | | |
| | II | 57* | 30 | 6.67 | 3.53 | 3.14 | 37.0 | 11.3 | 30.5 | 117 | | | | −0.020 |
| | III | 61* | 30 | 6.55 | 3.70 | 2.85 | 34.8 | 10.4 | 29.9 | 131 | 20.6 | 60 | 33 | −0.060 |
| | IV | 61 | | | | | | | | | | | | |
| | V | 55 | | 6.85 | 3.82 | 3.03 | 38.3 | 12.0 | 31.4 | 107 | | | | +0.740 |
| B.<br>6–5–50<br>4% | I | 134 | | 6.63 | 3.82 | 2.81 | 48.2 | 15.6 | 32.4 | 100 | | | | |
| | II | 119* | 30 | 6.79 | 4.40 | 2.39 | 40.0 | 12.9 | 32.3 | 140 | 28.8 | 101 | 23.1 | −0.405 |
| | III | 54* | 30 | | | | | | | | | | | |
| | IV | 56 | | 6.82 | 4.20 | 2.62 | 43.2 | 13.5 | 31.3 | 127 | | | | +0.420 |

* Period of infusion.

TABLE III

*The effect of infusion of hyperoncotic solutions of albumin on the concentrations of extracellular electrolytes, clearances, and rates of excretion of water and solutes in the urine*

| Study | Period | Duration | Extracell. $H_2O$ | | | Rates of excretion in urine | | | | | | Clear | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Na | Cl | K | $H_2O$ | Na | Cl | K | Urea | Total mOsm. | Creatin. | ea |
| | | *min.* | *mEq/L* | *mEq/L* | *mEq/L* | *cc./1°* | *mEq/1°* | *mEq/1°* | *mEq/1°* | *mM/1°* | *mM/1°* | *cc./min.* | |
| L. G. W.<br>10–11–48<br>25%—50 gm. | I | 118 | | 115 | | 92.4 | 12.6 | 14.4 | 4.5 | | | | |
| | II | 60* | | 113 | | 130.0 | 14.5 | 13.4 | 2.3 | | | | |
| | III | 60 | | 114 | | 352.0 | 12.7 | 13.1 | 2.6 | | | | |
| | IV | 60 | | 111 | | 65.0 | 13.2 | 15.1 | 3.8 | | | | |
| L. G. W.<br>10–18–48<br>25%—100 gm. | I | 120 | 146 | 115 | | 201.5 | 17.4 | 19.6 | 4.3 | 20.7 | 64.1 | 126 | 87.3 |
| | II | 57* | | | | 405 | 18.7 | 18.5 | 5.8 | 19.5 | 68.5 | 107 | 82.0 |
| | III | 78* | 146 | 111 | | 169.2 | 15.2 | 12.7 | 5.1 | 15.6 | 56.2 | 124 | 68.0 |
| | IV | 120 | 145 | 111 | | 141 | 17.8 | 18.6 | 2.5 | 16.5 | 57.1 | 113 | 75.0 |
| L. G. W.<br>Control for<br>10–18–48 | I | 120 | | | | 140 | 7.3 | 10.4 | | | | | |
| | II | 60* | | | | 380 | 15.9 | 20.5 | | | | | |
| | III | 60* | | | | 135 | 15.9 | 16.4 | | | | | |
| | IV | 120 | | | | 182 | 10.9 | 11.3 | | | | | |
| L. G. W.<br>12–11–48<br>25%—100 gm.<br>Dehydrated | I | 120 | 148 | 117 | 4.6 | 49.5 | 12.6 | 12.0 | 4.4 | | | 116.9 | |
| | II | 60* | | | | 58 | 12.9 | 9.8 | 6.3 | | | 106.1 | |
| | III | 60* | 150 | 116 | 4.2 | 51 | 9.5 | 6.3 | 6.4 | | | 102.5 | |
| | IV | 120 | 149 | 115 | 4.2 | 28.5 | 3.4 | 4.6 | 3.8 | | | 107.0 | |
| J. O.<br>10–15–48<br>25%—50 gm. | I | 120 | 144 | 115 | | 66 | 15.2 | 15.2 | 4.0 | | | | |
| | II | 60* | 147 | 115 | | 90 | 15.3 | 14.4 | 6.9 | | | | |
| | III | 60 | | | | 205 | 10.7 | 10.5 | 4.7 | | | | |
| | IV | 60 | 144 | 113 | | 158 | 16.1 | 16.6 | 1.7 | | | | |
| J. O.<br>10–20–48<br>25%—100 gm. | I | 120 | 141 | 111 | | 150 | 11.1 | 15.4 | 9.9 | 22.2 | 64.2 | 132 | 67.8 |
| | II | 63* | | | | 161.4 | 13.1 | 13.9 | 11.4 | 21.4 | 70.4 | 123 | 65.4 |
| | III | 58* | 143 | 112 | | 289.2 | 6.3 | 5.1 | 6.7 | 15.4 | 41.4 | 94 | 48.7 |
| | IV | 120 | 144 | 113 | | 292.5 | 7.1 | 4.9 | 3.1 | 16.1 | 36.5 | 112 | 50.8 |
| J. O.<br>1–8–49<br>10%—100 gm. | I | 120 | 142 | 116 | 4.2 | 61 | 11.6 | 14.6 | 7.0 | 17.6 | 54.8 | 132.1 | 58.5 |
| | II | 60* | | | | 78 | 8.5 | 11.1 | 12.1 | 15.8 | 57.0 | 121.2 | 54.7 |
| | III | 60* | 144 | 118 | 4.2 | 50 | 6.5 | 6.8 | 7.0 | 13.9 | 40.9 | 124.2 | 48.3 |
| | IV | 120 | 145 | 116 | 4.2 | 49 | 10.3 | 9.9 | 3.0 | 14.3 | 40.9 | 125.0 | 49.8 |
| J. O.<br>Control for<br>1–8–49 | I | 120 | | | | 58 | 11.3 | 13.1 | | | | | |
| | II | 60* | | | | 94 | 19.6 | 20.5 | | | | | |
| | III | 60* | | | | 126 | 25.0 | 26.3 | | | | | |
| | IV | 120 | | | | 145 | 29.3 | 29.2 | | | | | |
| R. N.<br>1–13–49<br>25%—100 gm. | I | 120 | 143 | 109 | 3.8 | 95 | 17.8 | 16.8 | 6.1 | 13.1 | 60.9 | 108.5 | 39.4 |
| | II | 60* | | | | 97 | 16.2 | 14.6 | 6.9 | 13.2 | 59.4 | 110.5 | 39.6 |
| | III | 60* | 142 | 110 | 3.6 | 70 | 7.2 | 6.6 | 7.6 | 10.9 | 40.5 | 102.3 | 38.8 |
| | IV | 120 | 143 | 108 | 3.7 | 71 | 9.1 | 7.9 | 5.6 | 11.1 | 40.5 | 102.5 | 39.1 |

* Period of infusion.

uresis of water occurred during the second hour of the infusion period and was always distinctly larger than the rate of excretion of water in the comparable period of the control study. This diuresis was not associated with a significant increase in the clearance of endogenous creatinine. Although the rates of excretion of sodium and chloride are also increased, they do not differ significantly from the rates of excretion of these ions in comparable periods of the control studies. The rate of excretion of potassium was increased when saline was the diluent for the albumin. The diuresis of water was not dependent on a decrease in the concentration of sodium in the extracellular water since it occurred with both 5% glucose and normal saline as the diluent.

In the post-infusion period there was a decrease in the rate of excretion of water and salt. This may be related to the fact that the large loss of water in the urine causes an increase in the concen-

TABLE IV

*infusion of hyperoncotic solutions of albumin on the concentrations of extracellular electrolytes, clearances, and of excretion of water and solutes in the urine in normal subjects with expanded extracellular volumes*

| | Period | Duration | Conc. in extracell. $H_2O$ | | | Rates of excretion in urine | | | | Clearance |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Na | Cl | K | $H_2O$ | Na | Cl | K | Creatinine |
| ii | | *min.* | *mEq/L* | *mEq/L* | *mEq/L* | *cc./1°* | *mEq/1°* | *mEq/1°* | *mEq/1°* | *cc./min.* |
| d n<br>f 13-50<br>2 —50 gm. | I | 249 | 145 | 118 | 3.9 | 60.7 | 17.3 | 15.6 | 3.0 | 99 |
| | II | 116* | 148 | 120 | 3.9 | 196.6 | 43.3 | 42.7 | 3.2 | 93.6 |
| | III | 89† | 147 | 120 | 3.5 | 357 | 63.9 | 60.8 | 4.8 | 97.8 |
| | IV | 72 | 148 | 120 | 3.8 | 210 | 37.3 | 35.9 | 2.8 | 86 |
| | V | 88 | 146 | 117 | 3.6 | 112.5 | 26.4 | 25.7 | 2.5 | 102 |
| B. B.<br>Control for<br>2-13-50 | I | 253 | | | | 37.5 | 10.9 | 9.3 | 2.4 | |
| | II | 123* | | | | 155 | 27.9 | 27.3 | 2.7 | |
| | III | 52‡ | | | | 397 | 54.5 | 53 | 4.8 | |
| | IV | 60 | | | | 525 | 76.6 | 77.2 | 8.1 | |
| | V | 109 | | | | 161.8 | 33.2 | 33.5 | 3.7 | |
| A.<br>12-14-49<br>25%—50 gm. | I | 62 | 147 | 114 | 4.3 | 91.8 | 10.5 | 12.7 | 6.5 | 163 |
| | II | 117* | 148 | 117 | 4.0 | 74.4 | 12.1 | 11.7 | 3.4 | 105.3 |
| | III | 56† | 146 | 115 | 3.8 | 128.8 | 21.8 | 21.0 | 4.2 | 100 |
| | IV | 63 | 147 | 118 | 4.0 | 123.9 | 20.8 | 19.2 | 3.6 | 97.2 |
| | V | 118 | 145 | 115 | 3.8 | 92.6 | 16.4 | 16.5 | 2.5 | 97.4 |
| A.<br>ontrol for<br>2-14-49 | I | 68 | | | | 68 | 10.4 | 14.2 | 5.7 | |
| | II | 119* | | | | 88.2 | 17.8 | 20.6 | 4.5 | |
| | III | 54‡ | | | | 138.6 | 31.8 | 32.2 | 3.6 | |
| | IV | 68 | | | | 113 | 27.9 | 27.5 | 2.8 | |
| | V | 119 | | | | 100.9 | 27.2 | 25.5 | 2.6 | |

eriod of infusion of 2,500 cc. normal saline.
eriod of infusion of 200 cc. 25% albumin.
eriod of infusion of 200 cc. normal saline.

tion of proteins in the serum similar to that observed with infusions of hyperoncotic solutions of albumin.

Infusions of 1,500 cc. of a 4% solution of albumin in saline containing 200 milli-units of a posterior pituitary preparation were unassociated with a diuresis of water, or any significant alteration in the rate of excretion of sodium, chloride, or potassium (Table VII).

## DISCUSSION

A review of the previous investigations on the alterations induced in the composition and rate of flow of urine promoted by increasing the volume of the plasma reveals many seemingly conflicting results. Knowlton (1) and Podhradszky (14) reported a diminished rate of flow of urine with infusions of solutions of colloid in contrast to saline in rabbits and dogs. However, Metcalf (15) and Orloff and Blake (16) reported an increase in the rate of excretion of water in dogs in response to infusions of plasma and concentrated human serum albumin.

Reports of the responses of the normal human subject to infusions of 25% salt-poor human albumin are also at variance. Goodyer, Peterson, and Relman (17) observed a reduction in the rate of excretion of water, sodium, and chloride with no significant change in the clearance of mannitol. More recently, Elkinton and his associates (2) described an increased rate of excretion of sodium in normal subjects with similar infusions. They also found no changes in the rate of glomerular filtration as measured by the clearance of mannitol, inulin, or endogenous creatinine. Cargill (3) infused normal hypertensive and nephritic subjects rapidly with 25% albumin and reported an increase in the clearance of inulin and PAH. It is impossible, at this time, to reconcile these conflicting results.

There may be less variability in the reported responses to iso-oncotic expansion of the plasma volume. Eggleton, Pappenheimer and Winton (4) compared the diuretic response in anesthetized dogs to an increase of glomerular filtration brought about by a 5 mm. Hg increase in capillary pressure,

TABLE V

*The effect of infusion of hyperoncotic solutions of albumin* at night *on the concentrations of extracellular electrolytes, clearances, and rates of excretion of water and solutes in the urine*

| Study | Period | Duration | Conc. in extracell. $H_2O$ | | | Rates of excretion in urine | | | | | | Clearances | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Na | Cl | K | $H_2O$ | Na | Cl | K | Urea | Total mOsm. | Creatin. | Urea |
| | | *min.* | *mEq/L* | *mEq/L* | *mEq/L* | *cc./1°* | *mEq/1°* | *mEq/1°* | *mEq/1°* | *mM/1°* | *mM/1°* | *cc./min.* | *cc./min.* |
| L. G. W.<br>10-25-48<br>25%—100 gm. | I | 120 | 143 | 112 | | 58 | 4.4 | 4.3 | 1.9 | 15.1 | 27.7 | 121 | 52.5 |
| | II | 59* | | | | 60 | 5.9 | 2.8 | 2.3 | 15.1 | 31.5 | 122 | 52.6 |
| | III | 61* | 144 | 111 | | 336 | 6.4 | 2.1 | 5.4 | 18.0 | 41.6 | 120 | 60.3 |
| | IV | 120 | 142 | 113 | | 221 | 4.9 | 3.5 | 2.6 | 16.6 | 31.6 | 106 | 57.6 |
| J. O.<br>2-1-49<br>25%—100 gm. | I | 120 | 146 | 112 | 4.5 | 26 | 4.8 | 5.4 | 3.2 | 11.9 | 27.9 | | |
| | II | 60* | | | | 38 | 6.0 | 5.4 | 3.9 | 14.4 | 34.2 | | |
| | III | 60* | 145 | 111 | 4.1 | 40 | 4.7 | 2.0 | 4.0 | 16.2 | 33.6 | | |
| | IV | 120 | 144 | 112 | 4.1 | 70 | 2.5 | 1.9 | 1.9 | 15.6 | 24.4 | | |
| J. O.<br>Control for<br>2-1-49 | I | 120 | | | | 23.5 | 2.6 | 3.7 | 2.6 | | | | |
| | II | 60* | | | | 46 | 6.9 | 7.4 | 4.8 | | | | |
| | III | 60* | | | | 30 | 3.7 | 3.2 | 2.3 | | | | |
| | IV | 120 | | | | 24 | 3.0 | 2.0 | 2.1 | | | | |

* Period of infusion.

TABLE VI

*The effect of infusion of hypo- and iso-oncotic solutions of albumin on the concentrations of extracellular electrolytes, clearances, and rates of excretion of water and solutes in the urine*

| Study | Period | Duration | Conc. in extracell. $H_2O$ | | | Rates of excretion in urine | | | | | | Clearances | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Na | Cl | K | $H_2O$ | Na | Cl | K | Urea | Total mOsm. | Creatin. | Urea |
| | | *min.* | *mEq/L* | *mEq/L* | *mEq/L* | *cc./1°* | *mEq/1°* | *mEq/1°* | *mEq/1°* | *mM/1°* | *mM/1°* | *cc./min.* | *cc./min.* |
| L. G. W.<br>1-12-49<br>4% albumin<br>in saline | I | 120 | 146 | 115 | 4.4 | 60.5 | 10.3 | 13.4 | 4.2 | 15.0 | 44.0 | 122.1 | 49.2 |
| | II | 60* | | | | 220 | 20.8 | 22.9 | 9.9 | 19.3 | 80.7 | 128 | 65.8 |
| | III | 60* | 148 | 121 | 4.4 | 810 | 41.4 | 39.2 | 10.4 | 17.0 | 120.6 | 124.5 | 58.1 |
| | IV | 120 | 150 | 122 | 4.4 | 208.5 | 31.2 | 33.7 | 7.6 | 14.4 | 92.0 | 115.5 | 50.5 |
| L. G. W.<br>2-17-49<br>5% albumin<br>in saline | I | 120 | 147 | 111 | 4.6 | 68 | 14.2 | 15.3 | 5.1 | | | | |
| | II | 60* | | | | 241 | 17.0 | 17.3 | 9.9 | | | | |
| | III | 60* | 148 | 115 | 4.0 | 460 | 24.3 | 22.7 | 10.3 | | | | |
| | IV | 120 | 149 | 115 | 4.3 | 200 | 20.3 | 21.6 | 7.3 | | | | |
| L. G. W.<br>2-24-49<br>6% albumin<br>in saline | I | 120 | 152 | 112 | 3.8 | 50.5 | 10.9 | 13.8 | 4.1 | | | | |
| | II | 60* | | | | 90 | 17.1 | 17.2 | 7.7 | | | | |
| | III | 60* | 151 | 116 | 3.9 | 570 | 30.9 | 24.2 | 9.6 | | | | |
| | IV | 120 | 154 | 118 | 3.9 | 177.5 | 11.5 | 13.9 | 5.6 | | | | |
| L. G. W.<br>Control for<br>4%, 5%, 6%<br>albumin | I | 120 | | | | 61 | 11.8 | 10.8 | 3.7 | | | | |
| | II | 60* | | | | 220 | 22.5 | 22 | 5.6 | | | | |
| | III | 60* | | | | 208 | 30.3 | 30.3 | 5.4 | | | | |
| | IV | 120 | | | | 282 | 49.6 | 54.8 | 10.2 | | | | |
| J. O.<br>1-22-49<br>4% albumin<br>in 5% glucose | I | 120 | 148 | 116 | 4.1 | 41 | 7.6 | 7.2 | 2.3 | 16.9 | 36.7 | 123 | |
| | II | 60* | 142 | 109 | 3.5 | 145 | 10.3 | 9.0 | 3.7 | 22.2 | 50.2 | 121 | |
| | III | 60* | 143 | 110 | 3.3 | 900 | 9.3 | 5.1 | 3.5 | 21.9 | 47.5 | 129.1 | |
| | IV | 120 | 146 | 112 | 4.1 | 300 | 10.5 | 9.1 | 2.5 | 17.5 | 43.5 | 110.5 | |
| J. O.<br>Control for<br>1-22-49 | I | 120 | 146 | 114 | 4.6 | 40 | 7.1 | 8.5 | 5.3 | | | | |
| | II | 60* | 138 | 111 | 4.1 | 45 | 7.4 | 8.6 | 5.9 | | | | |
| | III | 60* | 139 | 112 | 4.2 | 240 | 6.6 | 5.9 | 2.7 | | | | |
| | IV | 120 | 141 | 112 | 4.2 | 173 | 11.7 | 12.6 | 4.0 | | | | |

* Period of infusion.
Total volume of infusions—L. G. W.: 2,280 cc.; J. O.: 1,875 cc.

TABLE VII

*The effect of infusion of hypo-oncotic solutions of albumin on the concentrations of extracellular electrolytes, clearances, and rates of excretion of water and solutes in the urine under the influence of exogenous posterior pituitary hormone*

| Study | Period | Duration | Conc. in extracell. $H_2O$ | | | Rates of excretion in urine | | | | | | Clearance |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Na | Cl | K | $H_2O$ | Na | Cl | K | Urea | Total mOsm. | Creatinine |
| | | *min.* | *mEq/L* | *mEq/L* | *mEq/L* | *cc./1°* | *mEq/1°* | *mEq/1°* | *mEq/1°* | *mM/1°* | *mM/1°* | *cc./min.* |
| B. 6-5-50 | I | 119* | 142 | 117 | 4.0 | 79.2 | 17.6 | 18.4 | 9.5 | 20.9 | 75.1 | 105 |
| | II | 54 | | | | 53.3 | 12.2 | 12.3 | 4.6 | 13.6 | 67.2 | 103 |
| | III | 56 | 142 | 116 | 3.8 | 48.2 | 12.4 | 12.6 | 3.5 | 12.5 | 44.3 | 85 |
| B. Control for 6-5-50 | I | 116† | | | | 80.2 | 18.4 | 20.9 | 8.3 | 21.5 | 74.9 | |
| | II | 60 | | | | 65 - | 16.1 | 17.2 | 5.0 | 16.6 | 58.8 | |
| | III | 57 | | | | 89.5 | 24.1 | 23.9 | 4.5 | 20.4 | 77.6 | |
| S. 6-5-50 | I | 57* | 141 | 116 | 4.1 | 65.2 | 11.8 | 11.7 | 9.7 | 12.8 | 55.8 | 99.2 |
| | II | 61* | 141 | 117 | 3.9 | 65.9 | 14.9 | 10.7 | 8.2 | 12.0 | 58.2 | 104 |
| | III | 61 | | | | 56.0 | 14.7 | 12.5 | 4.9 | 13.1 | 52.3 | 99.6 |
| | IV | 55 | 142 | 117 | 4.1 | 306 | 13.5 | 14.4 | 4.5 | 19.1 | 55.1 | 95.5 |
| S. Control for 6-5-50 | I | 61† | | | | 88.5 | 23.9 | 21.2 | 7.9 | 16.3 | 79.9 | |
| | II | 60† | | | | 95.0 | 31.4 | 26.9 | 6.6 | 16.6 | 90.6 | |
| | III | 65 | | | | 83.0 | 28.0 | 33.1 | 4.2 | 15.3 | 79.7 | |
| | IV | 51 | | | | 241 | 26.5 | 26.8 | 4.9 | 18.9 | 81.7 | |

* Period of infusion of 1,500 cc. 4% albumin in normal saline with 200 milli-units post. pituitary hormone.
† Period of infusion of 1,500 cc. normal saline with 200 milli-units post. pituitary hormone.

and the equivalent increase in filtration induced by lowering the colloid osmotic pressure of the plasma. The infusion necessary to reduce the colloid osmotic pressure, of course, also increased the volume of the plasma and interstitial fluid. The diuresis induced by dilution was 15 times as great as that induced by the equivalent change in capillary pressure. They concluded that the dilution promoted specific changes in renal tubular function resulting in a decreased reabsorption of water. Wilson and Harrison (5) studied the effects of rapid infusions of 900–1,955 cc. of reconstituted human plasma in normal subjects. They observed an increase in the clearances of PAH and creatinine and a diuresis of water. The clearance of creatinine can hardly serve as a measure of the rate of glomerular filtration in this study since plasma levels were increased to 12–20 mgm.% by injection of this substance. Despite this, in three subjects to whom the infusions were administered more slowly (*i.e.*, 22.5–30 cc./min.) there was a diuresis of water in two with no alteration in the clearance of creatinine.

It is clear that the manner in which the volume of the plasma is increased and other more subtle variations may condition the response to this alteration in terms of the rates of excretion of water and electrolytes. In the present study there was a decrease in the rate of excretion of sodium, and sometimes water, in normal subjects when the plasma volume was expanded with concentrated solutions of albumin. These changes were not related to alterations in the rate of glomerular filtration as measured by the clearance of endogenous creatinine. An increase in the volume of the plasma achieved in this manner is, of course, associated with a contraction of the interstitial fluid volume and a rise in the colloid osmotic pressure of the plasma. In contrast to normal subjects, patients with the edema of the nephrotic syndrome (10–13) respond with increased rates of excretion of water and salt with this type of infusion despite the same changes in plasma volume, colloid osmotic pressure, and decrease in the volume of the interstitial fluid. There are, to be sure, quantitative differences in these alterations in the two groups. The volume of the interstitial fluid is contracted to an even greater extent and the increase in colloid osmotic pressure of the plasma is thereby mitigated in the edematous subjects. This is clearly demonstrated by comparing the increases in plasma volume expressed as cubic centimeters of plasma volume increase per gram of albumin infused in the two groups of subjects. The average increase was 20.6 cc. in the edematous patients (13), 13.2 cc. in the present study, and 9 cc. in the studies of Goodyer, Peterson, and Relman (17). Prior expansion of the extracellular volume with physiological saline in normal subjects did not modify the responses to infusions of hyperoncotic solutions of albumin; in these studies the average increase in plasma volume was 11.6 cc. per gm. of infused albumin. It seems unlikely, therefore, that the volume of the interstitial fluid *per se* influences the rates of excretion of sodium or water.

The question must be seriously raised as to whether a rise in plasma colloid osmotic pressure may be a stimulus for an increase in the reabsorption of salt and water by the renal tubules. Under ordinary circumstances an increase in the colloid osmotic pressure of the plasma is a reflection of dehydration associated with hemoconcentration. Greiner and Podhradszky (18) have suggested that a rise in plasma colloid pressure might activate the osmoreceptor mechanism of the supra-opticohypophyseal system. However, it is quite likely that the receptor organ for the posterior pituitary gland lies in an extravascular position. Moreover, if it were within the vascular tree it would be difficult to conceive that the minor contribution to a change in total osmotic pressure afforded by small variations in protein concentration could significantly influence this receptor. In addition, an antidiuresis does not always follow an infusion of a hyperoncotic solution of albumin, and when it does there is a reduction in the total milli-osmolar concentration of the urine. This is quite unlike what is observed with the antidiuresis promoted by the hormone of the posterior pituitary gland (19).

It is intriguing to speculate that a receptor organ lying within the vascular tree with the same characteristics of permeability as the endothelium could be specifically stimulated by the colloid (oncotic) pressure. Such an organ might be termed an *onco*-receptor. If the response to stimulation of such a mechanism were an increased reabsorption of sodium by the renal tubules, an increased reabsorption of water might follow as a consequence.

The retention of sodium and chloride associated with infusions of concentrated albumin appears not to be mediated through the action of the adrenal cortex. Subject R.N. is a patient with Addison's disease of 12 years' standing. He did not respond with an eosinopenia nor an increase in the excretion of 17-ketosteroids in the urine following large doses of ACTH. An infusion of 25% albumin was associated with a distinct decrease in the rates of excretion of sodium and chloride in this patient (Table III).

There was no decrease in the rates of excretion of sodium associated with the infusion in normal subjects of hyperoncotic solutions of albumin in the evening studies (Table V). The rates of excretion of sodium and chloride in the control period were lower in the evening than in the morning studies, which is a reflection of the diurnal variation in mineral excretion (20, 21). There is, as yet, no valid explanation for the diurnal variation. However, this difference in response to an infusion of concentrated albumin at two different times of day emphasizes the importance of controlling this factor in studies involving the metabolism of electrolytes and water.

The increase in plasma volume associated with infusions of hypo- and iso-oncotic solutions of albumin promoted a striking augmentation in the rate of excretion of water with no essential change in the rates of excretion of sodium and chloride, when compared with the control studies. There is no evidence in these studies that this diuresis need be associated with an increase in the rate of glomerular filtration. Moreover, the diuresis of water was independent of the concentration of sodium in the serum and the rate of excretion of this ion. The fact that there was an antidiuresis following an infusion of a liter of 10% albumin militates against the possibility that the diuresis observed with hypo- and iso-oncotic solutions of albumin is due to a dilution of circulating posterior pituitary antidiuretic hormone. The lack of a diuresis associated with infusions of 25% and 10% solutions of albumin rejects the possibility that the albumin might inactivate pituitary antidiuretic hormone.

There remain two alternative explanations. An expansion of the plasma volume unassociated with any other measured alteration may be a direct stimulus for decreased reabsorption of water by the renal tubules, or it may, in some manner, suppress the production of antidiuretic hormone by the posterior pituitary gland. The absence of the usual diuretic response to an infusion of a hypo-oncotic solution of albumin when exogenous posterior pituitary hormone is incorporated in the infusate is compatible with the latter explanation.

It may be possible to explain the different responses in the excretion of water and salt to infusions of hyperoncotic solutions of albumin in the normal subject and the patient with the nephrotic syndrome. The response to expansion of the plasma volume *per se* appears to be a diuresis of water. However, when the expansion of the plasma volume is achieved in the normal subject with a hyperoncotic solution of albumin, the diuresis of water is prevented, perhaps, by the associated increase in colloid osmotic pressure of the plasma which promotes an increased reabsorption of sodium, and consequently, water. In the nephrotic, on the other hand, the increase in plasma volume is greater, the rise in colloid osmotic pressure is thereby mitigated, and since tubular reabsorption of sodium is already nearly maximal, it can not be enhanced. Since tubular reabsorption of sodium can not be increased very much, the stimulus for water diuresis afforded by the expanded plasma volume is not inhibited.

In another investigation (13) it was demonstrated that the increase in the rate of excretion of sodium in the nephrotic associated with the infusion of a hyperoncotic solution of albumin occurred when the concentration of sodium in the extracellular water was increased as a consequence of the initial diuresis of water. The diuresis of water in the normal subject associated with infusion of an iso-oncotic solution of albumin is not followed by an increase in the rate of excretion of sodium. This may result from the fact that the large excretion of water causes an increase in the concentration of protein in the serum. This rise in colloid osmotic pressure promotes an increase in the tubular reabsorption of sodium and secondarily terminates the diuresis of water.

The present investigations are obviously inconclusive concerning the mechanisms responsible for the observed responses to infusions of hyper- and iso-oncotic solutions of albumin. Further work is in process in an attempt to define these mechanisms. The working hypotheses are that uncomplicated expansion of the plasma volume initiates a diuresis of water by suppressing the activity of the posterior pituitary gland, and that an acute increase in the colloid osmotic pressure of the plasma leads to an increase in renal tubular reabsorption of sodium through some unknown mechanism.

## SUMMARY AND CONCLUSIONS

1. The intravenous administration of hyperoncotic solutions of albumin to normal human subjects in the morning results in a decreased rate of excretion of sodium and chloride which is sometimes associated with a decreased rate of excretion of water.

2. The decrease in rates of excretion of sodium and chloride is due, *presumably, to an increase in* renal tubular reabsorption of these ions, since there were no significant alterations in the clearance of endogenous creatinine. This increase in renal tubular reabsorption of salt is apparently not mediated via the adrenal cortex, since it was clearly observed in a subject with well validated Addison's disease.

3. The increase in plasma volume resulting from infusions of hyperoncotic solutions of albumin is derived from the interstitial fluid. However, the magnitude of the increase in plasma volume is not sufficient to prevent a significant increase in the concentration of albumin in the serum. The resultant increase in colloid osmotic pressure of the plasma *may* be the stimulus that promotes the increased reabsorption of sodium and chloride. An increase in oncotic pressure could be an effective stimulus if there were an onco-receptor which resided within the vascular tree.

4. These phenomena are not observed when the infusions of hyperoncotic solutions of albumin are administered in the evening. The differences between the morning and evening studies may be related to the diurnal variation in the rates of excretion of water and electrolytes. The modifying influence of the time of day on these experiments emphasizes the importance of considering the various circumstances that may condition a response with respect to the metabolism of electrolytes and water.

5. Experimentally induced expansion of the extracellular volume by approximately 2 liters does not modify the responses to the infusion of a hyperoncotic solution of albumin.

6. Infusion of iso- and hypo-oncotic solutions of albumin promotes a striking diuresis of water with no alteration in the rate of excretion of salt or clearance of endogenous creatinine. This diuresis appears to be due to a decreased reabsorption of water in the renal tubule. It is suggested

that simple iso-oncotic expansion of the plasma volume suppresses the secretion of the posterior pituitary antidiuretic hormone.

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# THE DETERMINATION OF SULFHYDRYL GROUPS IN SERUM. II. PROTEIN ALTERATIONS ASSOCIATED WITH DISEASE[1]

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(Submitted for publication February 14, 1951, accepted April 23, 1951)

## INTRODUCTION

The status of the serum proteins both in health and disease has been extensively studied in the hope that data might be assembled which would contribute to knowledge of the pathogenesis of many diseases, aid in the classification of the metabolic alterations induced by pathological states, be of use in early diagnosis or differential diagnosis, and serve as a rationale for improved supportive therapy (1). Ever since glutathione was isolated from yeast by Hopkins (2), increasing attention has been devoted to studies of the role played by the sulfhydryl group in enzyme activity (3–6), cell division and growth (7, 8) and protein structure (9–11).

Because of the importance of its biochemical associations, an investigation of the sulfhydryl group was undertaken as part of a program for the study of the mechanism of action of various chemical agents on the growth and viability of normal and malignant cells. Blood was selected initially for this study because, *a*) to a certain extent, it is in equilibrium with and reflects the status of other body tissues; *b*) it has been exhaustively studied; and *c*) a uniform sample can be obtained from an individual.

A preliminary study showed that the sulfhydryl content of the serum protein from normal individuals was relatively constant, and the distribution was within a narrow range. The values were reduced when the sera from patients suffering from neoplastic, infectious or metabolic diseases were similarly examined (12). The relationship of the serum protein components to their content of sulfhydryl groups was then examined. The sulfhydryl content of normal serum, serum albumin, and serum globulin was determined with respect to the total nitrogen and peptide content of these fractions (13). These determinations revealed that normal serum and its fractions could be distinguished by characteristic relationships between the sulfhydryl group and the total nitrogen or the number of peptide bonds in the proteins. The present report will describe similar observations on sera from patients with various diseases.

## METHODS AND MATERIALS

The details for obtaining the serum sample, storage, fractionation, nitrogen analysis, biuret analysis, sulfhydryl determination and calculations have been published (13). In summary, the procedures employed were:

1. Blood was obtained from patients in the fasting state and the serum carefully separated to avoid hemolysis. The serum was either examined in the fresh state soon thereafter or stored at minus 78°C until analyzed.
2. The serum was fractionated according to the method of Pillemer and Hutchinson (14).
3. The protein content of the serum and the albumin and globulin components were determined by the Kjeldahl nitrogen and biuret methods. The term D, when employed, refers to the optical density in the biuret method. Electrophoretic analyses were also performed in veronal buffer pH 8.6, 0.1 ionic strength, employing a modified Longsworth apparatus. The descending mobility was routinely employed except as noted with individual sera. Components migrating with a mobility of 2.0 to $2.5 \times 10^{-5}$ cm$^2$/sec$^{-1}$/volt$^{-1}$ have been designated as M protein. Sera which contained pigment, lipid or other interfering components which rendered them unsuitable for electrophoretic analysis are not included. No attempt was made to remove these interfering substances by filtration, extraction, freezing, etc. They are designated as unsatisfactory samples or not done.
4. The sulfhydryl determinations were made by amperometric titration in 21% methanol solution containing ammonium nitrate and ammonium hydroxide as supporting electrolytes. The current which flowed between a mercury-mercuric iodide electrode and a rotating platinum electrode was measured with a sensitive galvanometer. Duplicate determinations on individual sera showed less

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[1] This investigation was conducted under a grant from the American Cancer Society to the Department of Preventive Medicine, The Johns Hopkins University School of Medicine, recommended by the Committee on Growth of the National Research Council.

than a 2% variation. The results are expressed as micromoles of sulfhydryl (SH). One ml. of .001 N silver nitrate is equivalent to 1 micromole of SH.

The sulfhydryl content of the serum and its constituents is expressed as follows:

a) *The sulfhydryl content of the total protein in 100 ml whole serum or of the albumin and globulin components contained in that volume.*

b) *The sulfhydryl content per gram of Kjeldahl nitrogen obtained employing whole serum, albumin, or globulin fractions.* No correction was made for the serum N.P.N., as among the normals the latter represented approximately 3% of the total nitrogen and, even in the abnormal states studied, the final values (with few exceptions) were not apparently influenced by such correction.

c) *The sulfhydryl content per unit of optical density with a biuret method.* This has been utilized to characterize the protein with respect to its peptide content. The total nitrogen determination measures peptide nitrogen, non-peptide amino acid nitrogen such as ϵ amino, imidazole, or guanidinium nitrogen as well as the non-protein nitrogenous constituents of serum. The quantitative biuret determination is influenced only by the number of nitrogen atoms bound in peptide linkage. Human serum albumin has been found to contain approximately 577 peptide bonds per mole (15). The 586 amino acids probably exist as nine polypeptide chains each of which contains approximately 65 amino acids. These polypeptide chains lie adjacent to each other and are bound to each other by various types of cross linkages, one of which presumably yields the sulfhydryl group when the albumin molecule is titrated amperometrically. We have found that serum albumin from normal individuals contained 0.71 mole of SH per mole of albumin. This was believed to represent the mean resulting from the presence of a mercaptalbumin containing 1 mole of (SH) sulfhydryl per mole of protein and an albumin molecule containing no amperometrically detectable sulfhydryl (13). We wish to emphasize that it is easier to detect a quantitative change in the single sulfhydryl group per albumin molecule than greater changes in the 577 peptide bonds. Thus, the functional significance of a single sulfhydryl group for enzyme activity may be related to the importance of this group in protein structure (5). For these reasons, it was decided to study the relationship of the sulfhydryl content of the serum proteins with respect to their total nitrogen and peptide content. Electrophoretic analyses provide information with respect to the relative distribution of the recognized major protein components of sera.

## RESULTS

The normal values for the serum protein sulfhydryl content have been presented in detail. No significant difference with respect to age or sex was observed (13). In order to compare the data from patients to be described, a summary of the normal values is presented in Table I. The variation among these normal individuals was quite small as indicated by the range and the standard error for these determinations. The results of the preliminary survey of patients' sera are summarized in Table II (12). While the sulfhydryl values differed significantly from the normal, no differential characteristic for the individual groups was apparent.

It was therefore deemed desirable to investigate a group of patients more intensively so as to establish the nature of the sulfhydryl abnormalities observed. Because of individual variation in disease, it did not appear feasible to treat the observations in this series statistically. Each observation will be presented in detail. The data on the various classes of patients whose sera were subjected to such detailed analysis are recorded in Tables III–VI.

### *Malignant Tumors*

In Table III, the individual determinations on 11 patients with malignant tumors are presented. The diagnosis in all cases was established by biopsy, operation, and in some cases by autopsy. Nine of the 11 patients had evidence of carcinoma with multiple metastatic involvement. One patient (No. 9) had a rhabdomyosarcoma with pulmonary metastases and one (No. 3) had a carcinoma localized to the breast. The sera were obtained more than two months before exitus with the exception of the second specimen from patient 4, dated 5/13/49, and that from patient 10, dated 7/12/49, which were drawn within one week preceding their death.

As may be noted in Table III, and subsequent tables, the total nitrogen content of the serum protein is a poor index of the relative concentrations of albumin and globulin. The total nitrogen content did not deviate markedly from the normal, although the individual albumin and globulin values were distinctly abnormal (col. 4). The albumin content was below normal in the sera of all patients except one (patient 8, 12/12/49). The sulfhydryl determination on the unfractionated serum, unlike the total nitrogen values, was reduced in almost all instances (col. 5). The two exceptions were serum obtained on 6/8/50 from patient 9, while in an A-Methopterin induced remission, and the serum from patient 11 with a melanocarcinoma.

TABLE I

*Summary of normal serum protein and sulfhydryl values*

| DETERMINATION | MALES | | | FEMALES | | |
|---|---|---|---|---|---|---|
| | RANGE | MEAN | ± 1 S.E. | RANGE | MEAN | ± 1 S.E. |
| TOTAL N AS Gr. PER 100 ml SERUM | | | | | | |
| SERUM | (1.09-1.26) | 1.20 | .02 | (1.13-1.24) | 1.18 | .01 |
| ALBUMIN | (.64-.78) | .71 | .01 | (.65-.73) | .69 | .01 |
| GLOBULIN | (.37-.51) | .45 | .02 | (.40-.54) | .47 | .01 |
| MICROMOLES SH PER 100 ml SERUM | | | | | | |
| SERUM | (52.0-59.0) | 53.9 | .7 | (48.8-55.8) | 52.6 | .7 |
| ALBUMIN | (39.6-47.8) | 44.9 | .9 | (40.1-47.4) | 43.9 | .8 |
| GLOBULIN | (9.2-12.3) | 10.9 | .4 | (9.9-12.5) | 10.8 | .3 |
| MICROMOLES SH PER GRAM N. | | | | | | |
| SERUM | (42.2-47.7) | 45.1 | .7 | (39.3-47.3) | 44.3 | .8 |
| ALBUMIN | (59.5-69.4) | 63.6 | 1.0 | (59.0-67.2) | 64.0 | .8 |
| GLOBULIN | (22.2-27.3) | 24.6 | .5 | (21.0-26.0) | 23.2 | .5 |
| D* PER mg N | | | | | | |
| SERUM | (.228-.258) | .243 | .003 | (.233-.261) | .243 | .003 |
| ALBUMIN | (.234-.261) | .244 | .003 | (.231-.256) | .239 | .003 |
| GLOBULIN | (.254-.290) | .265 | .004 | (.258-.282) | .267 | .003 |
| MICROMOLES SH PER D* PER 100 ml SERUM | | | | | | |
| SERUM | (.164-.198) | .186 | .004 | (.165-.203) | .181 | .004 |
| ALBUMIN | (.237-.283) | .261 | .006 | (.248-.283) | .266 | .004 |
| GLOBULIN | (.085-.103) | .093 | .002 | (.081-.098) | .088 | .006 |
| ALBUMIN-GLOBULIN RATIO | | | | | | |
| KJELDAHL | (1.26-1.97) | 1.61 | .08 | (1.22-1.83) | 1.47 | .06 |
| BIURET | (1.26-1.84) | 1.48 | .07 | (1.09-1.60) | 1.32 | .05 |

D* = OPTICAL DENSITY FOR THE BIURET (PEPTIDE) DETERMINATION

TABLE II

*Summary of values for serum sulfhydryl in patients with disease*

| DISEASE | NO. OF DETERMIN-ATIONS | $\mu$MOLES SH/100 ml SERUM | | | $\mu$ MOLES SH/G. NITROGEN | | |
|---|---|---|---|---|---|---|---|
| | | RANGE | MEAN | ± 1 S.D. | RANGE | MEAN | ± 1 S.D. |
| 1. CANCER | 46 | 25.3 - 43.6 | 35.4 | 1.9 | 22.1 - 35.6 | 31.7 | 4.0 |
| 2. LEUKEMIA | 34 | 20.4 - 47.0 | 37.7 | 5.4 | --- --- | --- | --- |
| 3. INFECTION | 13 | 28.7 - 50.0 | 41.6 | 5.2 | 24.1 - 42.7 | 34.4 | 5.0 |
| 4. DEGENERATIVE DISEASE | 12 | 24.0 - 49.6 | 40.7 | 7.8 | 21.0 - 38.0 | 31.8 | 6.1 |
| 5. MISCELLANEOUS | 12 | 8.6 - 37.5 | 22.3 | 7.5 | 8.4 - 33.5 | 22.8 | 8.0 |

1. CARCINOMA, SARCOMA, MELANOCARCINOMA, ETC.
2. LEUKEMIA AND OTHER LYMPHOMAS.
3. INFECTION INCLUDED LOBAR PNEUMONIA, ATYPICAL PNEUMONIA, TUBERCULOSIS, MENINGITIS, MEASLES, ETC.
4. DEGENERATIVE DISEASE INCLUDED ASTHMA, DIFFUSE ARTERIOSCLEROSIS WITH HYPERTENSION, HYPERTROPHIC ARTHRITIS, AND GOUT.
5. CIRRHOSIS OF THE LIVER, CHRONIC NEPHRITIS, ACUTE LUPUS ERYTHEMATOSUS DISSEMINATA, HEMACHROMATOSIS, IN WHICH ABNORMAL ALBUMIN AND GLOBULIN PATTERNS WERE PRESENT.

TABLE III

*Protein, sulfhydryl and electrophoretic data of sera from patients with carcinoma*

| 1<br>PAT. | 2<br>AGE & SEX | 3<br>DIAGNOSIS | 4<br>TOTAL NITROGEN AS G./100 ml SERUM | | | 5<br>μMOLES SH PER 100 ml SERUM | | | 6<br>μMOLES SH PER G. OF NITROGEN | | | 7<br>D∞ PER mg NITROGEN | | | 8<br>μMOLES SH PER D 100 ml SERUM | | | 9<br>ALBUMIN / GLOBULIN | | | | 10<br>PERCENT | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | SERUM | ALB. | GLOB. | SERUM | ALB. | GLOB. | SERUM | ALB. | GLOB. | SERUM | ALB. | GLOB. | SERUM | ALB. | GLOB. | HOWE | KJFLD. | BIUR. | ELEC. | ALB. | α1 | α2 | β | M | γ |
| 1 | 42<br>F | CA. RECTUM WITH METASTASIS<br>1-5-49 | 1.05 | .49 | .48 | 35.0 | 27.0 | 10.7 | 33.3 | 55.1 | 22.3 | N.D. | | | N.D. | | | 1.92 | 1.02 | | | N.D. | | | | | |
| 2 | 50<br>F | CA. OVARIAN NEOPLASM<br>9-28-49 | 1.03 | .46 | .53 | 29.8 | 21.9 | 7.0 | 28.9 | 47.6 | 13.2 | .241 | .240 | .267 | .121 | .197 | .049 | 2.1 | 0.87 | 0.78 | 0.54 | 35 | 16 | 21 | 17 | -- | 11 |
| 3 | 63<br>F | CA. BREAST<br>2-21-49 | 1.19 | .55 | .60 | 38.9 | 34.5 | 9.2 | 32.7 | 62.8 | 15.3 | .259 | .283 | .259 | .126 | .221 | .058 | 2.0 | 0.92 | 0.99 | 0.76 | 43 | 10 | 12 | 17 | -- | 18 |
| | | AMINOPTERIN<br>5-11-49 | 1.25 | .61 | .57 | 44.0 | 30.5 | 11.1 | 35.2 | 50.0 | 19.3 | .245 | .248 | .265 | .144 | .201 | .074 | 2.1 | 1.07 | 1.01 | 0.92 | 48 | 7 | 10 | 18 | -- | 17 |
| 4 | 46<br>F | CA. OVARY WITH METASTASIS<br>2-21-49 | 0.98 | .39 | .55 | 31.9 | 21.0 | 13.0 | 32.5 | 53.9 | 23.6 | .260 | .256 | .310 | .125 | .212 | .076 | 1.7 | 0.70 | 0.58 | | ICTERIC | | | | | |
| | | A-METHOP.<br>5-13-49 | 0.95 | .48 | .43 | 27.2 | 20.8 | 8.6 | 28.6 | 43.3 | 20.0 | .257 | .271 | .276 | .111 | .160 | .072 | 1.9 | 1.12 | 1.09 | 0.56 | 36 | 11 | 13 | 25 | -- | 15 |
| 5 | 47<br>F | CA. BREAST WITH METASTASIS<br>9-13-49 | 1.28 | .58 | .59 | 43.0 | 34.8 | 12.8 | 33.6 | 60.0 | 21.7 | .223 | .211 | .241 | .151 | .285 | .090 | 2.0 | 0.98 | 0.86 | 0.75 | 43 | 6 | 13 | 19 | -- | 19 |
| 6 | 63<br>F | CA. BREAST WITH METASTASIS<br>6-27-49 | 0.95 | .43 | .47 | 20.7 | 15.2 | 7.4 | 21.8 | 35.4 | 15.7 | .264 | .260 | .291 | .083 | .137 | .055 | 1.8 | 0.91 | 0.82 | 0.54 | 35 | 12 | 15 | 23 | -- | 15 |
| 7 | 47<br>F | CA. BREAST WITH METASTASIS<br>2-21-49 | 0.94 | .44 | .45 | 30.8 | 24.2 | 10.0 | 32.8 | 55.0 | 22.2 | .247 | .262 | .277 | .132 | .212 | .031 | 2.0 | 0.97 | 0.92 | | N.D. | | | | | |
| | | A-METHOP.<br>6-29-49 | 0.91 | .51 | .40 | 31.5 | 26.3 | 8.0 | 34.6 | 51.6 | 20.0 | .234 | .222 | .252 | .148 | .233 | .079 | | 1.28 | 1.12 | 1.17 | 54 | 9 | 14 | 12 | -- | 11 |
| 8 | 48<br>F | CA. BREAST WITH METASTASIS & PLEURAL EFFUSION<br>12-12-49 | 1.23 | .69 | .72 | 25.3 | 17.2 | 9.6 | 20.6 | 24.9 | 13.3 | .242 | .183 | .262 | .085 | .135 | .051 | 1.9 | 0.97 | 0.68 | 0.70 | 41 | 10 | 15 | 19 | -- | 15 |
| | | A-METHOP.<br>1-17-50 | 0.94 | .42 | .51 | 33.9 | 23.5 | 16.2 | 36.1 | 56.0 | 31.8 | .257 | .263 | .273 | .140 | .214 | .117 | | 0.82 | 0.79 | | N.D. | | | | | |
| 9 | 43<br>M | RHABDOMYO-SARCOMA WITH PULMONARY METASTASIS<br>4-24-50 | 1.22 | .47 | .74 | 27.0 | 22.4 | 8.8 | 22.1 | 47.7 | 11.9 | .245 | .247 | .245 | .090 | .193 | .049 | 1.61 | 0.64 | 0.64 | | N.D. | | | | | |
| | | A-METHOP.<br>6-8-50 | 1.34 | .58 | .72 | 52.0 | 37.6 | 17.0 | 38.8 | 64.8 | 23.6 | .258 | .257 | .282 | .150 | .252 | .084 | 1.22 | 0.73 | 0.81 | 0.92 | 48 | 7 | 8 | 17 | --- | 20 |
| | | 7-6-50 | 1.26 | .52 | .69 | 40.8 | 23.1 | 13.0 | 32.4 | 44.2 | 18.7 | .259 | .256 | .279 | .125 | .172 | .067 | | 0.75 | 0.69 | 0.89 | 47 | 7 | 9 | 17 | --- | 20 ASC |
| | | 7-31-50 | 1.25 | .44 | .74 | 31.0 | 21.0 | 14.0 | 24.8 | 47.6 | 19.0 | .240 | .240 | .268 | .103 | .198 | .071 | | 0.60 | 0.54 | 0.73 | 42 | 10 | 13 | 13 | --- | 22 |
| 10 | 52<br>M | MELANOCAR-CINOMA WITH METASTASIS<br>7-12-49 | 1.08 | .41 | .59 | 31.9 | 20.5 | 10.4 | 29.5 | 50.0 | 17.6 | N.D. | | | N.D. | | | | 0.70 | --- | 0.43 | 30 | 17 | 22 | 18 | --- | 13 |
| 11 | 60<br>M | MELANOCAR-CINOMA WITH METASTASIS<br>3-21-50 | 1.24 | .61 | .64 | 57.3 | 42.7 | 21.6 | 46.2 | 70.0 | 33.8 | .253 | .251 | .252 | .182 | .277 | .133 | 1.8 | 0.95 | 0.95 | 0.85 | 46 | 6 | 10 | 16 | --- | 22 |

D∞ = OPTICAL DENSITY

N.D. - NOT DONE

-- NOT PRESENT

TABLE IV

*Protein, sulfhydryl and electrophoretic data of sera from patients with multiple myeloma*

| 1 PAT. | 2 AGE & SEX | 3 DATE | 4 TOTAL NITROGEN AS G./100 ml SERUM | | | 5 μMOLES SH PER 100 ml SERUM | | | 6 μMOLES SH PER G. OF NITROGEN | | | 7 D* PER mg NITROGEN | | | 8 μMOLES SH PER D 100 ml SERUM | | | 9 ALBUMIN / GLOBULIN | | | | 10 PERCENT | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | SERUM | ALB. | GLOB. | SERUM | ALB. | GLOB. | SERUM | ALB. | GLOB. | SERUM | ALB. | GLOB. | SERUM | ALB. | GLOB. | HOWE | KJELD. | BIUR. | ELEC. | ALB. | α1 | α2 | β | Mα | γ |
| 1 | 67 | 11/25/49 | 1.63 | 0.37 | 1.20 | 24.9 | 16.8 | 10.5 | 15.3 | 45.4 | 8.7 | .273 | .240 | .292 | .056 | .189 | .030 | .42 | .31 | .25 | | UNSATISFACTORY | | | | | |
| | P | 12/8/49 | 1.52 | 0.28 | 1.13 | 19.9 | 11.7 | 9.2 | 12.4 | 41.8 | 7.8 | .259 | .239 | .294 | .048 | .175 | .027 | .41 | .24 | .19 | .25 | 20 | 6 | 10 | 8 | 56 | -- |
| 2 | 62<br>F | 3/7/50 | 2.06 | 0.15 | 1.84 | 75.8 | 11.9 | 74.0 | 36.9 | 78.8 | 40.2 | .247 | .238 | .286 | .150 | .331 | .146 | .28 | .08 | .07 | .12 | 11 | 5 | 5 | -- | 22 | 57 |
| 3 | 62 | 2/7/50 | 1.38 | 0.11 | 1.23 | 7.2 | 0.0 | 7.0 | 5.2 | | 5.7 | .227 | | .239 | .023 | | .024 | .25 | .09 | .06 | .25 | 20 | 7 | 6 | 8 | -- | 59 |
| | M | 3/6/50 | 1.21 | 0.22 | 0.97 | 14.1 | 9.8 | 8.6 | 11.6 | 45.4 | 8.9 | .243 | .245 | .264 | .048 | .185 | .034 | .54 | .22 | .21 | .37 | 27 | 7 | 10 | 9 | -- | 47 |
| 4 | 54<br>M | 3/15/50 | 1.80 | 0.32 | 1.43 | 22.0 | 14.2 | 12.4 | 12.2 | 44.9 | 8.7 | .250 | .234 | .249 | .049 | .192 | .035 | .56 | .22 | .21 | .32 | 24 | 6 | 10 | -- | 11 | 49 |
| 5 | 57<br>M | 3/15/50 | 1.64 | 0.35 | 1.53 | 55.0 | 19.6 | 42.8 | 33.9 | 56.8 | 28.0 | .254 | .238 | .244 | .133 | .239 | .115 | .70 | .23 | .22 | .30 | 23 | 5 | 8 | 14 | 42 | 8 |
| 6 | 51<br>F | 4/29/50 | 0.93 | 0.45 | 0.48 | 26.0 | 19.8 | 8.4 | 28.0 | 43.9 | 17.7 | .235 | .235 | .244 | .199 | .187 | .072 | 2.3 | .95 | .91 | .61 | 38 | 18 | 18 | 17 | -- | 9 |
| 7 | 48<br>M | 4/27/50 | 1.95 | 0.34 | 1.52 | 24.0 | 17.2 | 8.6 | 12.3 | 51.1 | 5.6 | .250 | .225 | .269 | .049 | .226 | .022 | .55 | .22 | .19 | | UNSATISFACTORY | | | | | |
| 8 | 64 | 2/24/50 | 1.17 | 0.28 | 0.89 | 31.3 | 15.2 | 16.4 | 26.9 | 55.1 | 18.5 | .256 | .254 | .260 | .105 | .217 | .071 | .64 | .31 | .30 | .35 | 26 | 7 | 13 | 15 | -- | 39 |
| | M | 3/8/50 | | | | | | | | | | | | | | | | | | | .41 | 29 | 8 | 15 | 14 | -- | 34 |

D* - OPTICAL DENSITY

Mα - MOBILITY OF FRACTION BETWEEN 2.0 AND 2.5 x $10^{-5}$ $cm^2 v^{-1} sec^{-1}$.

-- NOT PRESENT

TABLE V

*Protein, sulfhydryl and electrophoretic data of sera from patients with lupus erythematosus disseminata*

| 1 PAT. | 2 AGE & SEX | 3 DATE | 4 TREATMENT | 5 TOTAL NITROGEN AS G./100ml SERUM | | | 6 μMOLES SH PER 100 ml SERUM | | | 7 μMOLES SH PER G. OF NITROGEN | | | 8 D* PER mg NITROGEN | | | 9 μMOLES SH PER D 100 ml SERUM | | | 10 ALBUMIN / GLOBULIN | | | | 11 PERCENT | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | SERUM | ALB. | GLOB. | SERUM | ALB. | GLOB. | SERUM | ALB. | GLOB. | SERUM | ALB. | GLOB. | SERUM | ALB. | GLOB. | HOWE | KJELD. | BIUR. | ELEC. | ALB. | α1 | α2 | β | M | γ |
| 1 | 35 | 4/29/49 | | 1.13 | 0.27 | 0.80 | 25.8 | 14.7 | 12.3 | 22.8 | 54.4 | 15.4 | .256 | .244 | .273 | .089 | .223 | .056 | 0.54 | 0.34 | 0.30 | | N.D. | | | | | |
| | F | 5/6/49 | ALB. | 1.13 | 0.37 | 0.73 | 27.7 | 18.9 | 13.4 | 24.5 | 51.1 | 18.4 | .274 | .268 | .307 | .089 | .191 | .060 | 1.1 | 0.51 | 0.44 | | N.D. | | | | | |
| 2 | 31 | 5/2/49 | | 1.05 | 0.24 | 0.71 | 14.2 | 6.8 | 8.6 | 13.5 | 28.3 | 12.1 | .249 | .200 | .275 | .054 | .142 | .044 | | 0.34 | 0.25 | | N.D. | | | | | |
| | F | 5/6/49 | | 1.02 | | | 8.6 | | | 8.4 | | | .236 | | | .036 | | | | | | | N.D. | | | | | |
| 3 | 26<br>F | 5/4/49 | | 1.16 | 0.39 | 0.70 | 26.3 | 18.7 | 11.7 | 22.7 | 47.9 | 16.7 | .253 | .254 | .281 | .088 | .189 | .059 | | 0.56 | 0.50 | | N.D. | | | | | |
| 4 | 16 | 7/30/49 | | 1.75 | 0.05 | 1.70 | 14.5 | 0.0 | 13.6 | 8.3 | | 8.0 | .266 | | .269 | .031 | | .030 | | | | 0.22 | 18 | 4 | 5 | -- | 11 | 62 |
| | F | 8/11/49 | ACTH | 1.35 | 0.24 | 1.04 | 21.1 | 9.1 | 13.6 | 15.6 | 37.9 | 13.1 | .245 | .233 | .269 | .064 | .163 | .049 | 0.72 | 0.23 | 0.20 | 0.39 | 28 | 7 | 8 | 12 | -- | 45 |
| | | 9/20/49 | ACTH | 1.19 | 0.36 | 0.75 | 33.5 | 20.3 | 12.0 | 28.2 | 56.4 | 16.0 | .242 | .258 | .284 | .116 | .221 | .056 | 1.25 | 0.48 | 0.44 | 0.79 | 44 | 8 | 9 | 14 | -- | 25 |
| 5 | 45 | 6/16/49 | ACTH | 0.88 | 0.44 | 0.42 | 27.9 | 20.8 | 8.7 | 31.7 | 47.3 | 20.7 | .242 | .241 | .264 | .131 | .196 | .078 | | 1.05 | 0.95 | 0.72 | 42 | 9 | 15 | 20 | -- | 14 |
| | M | 8/10/49 | ACTH | 1.05 | 0.53 | 0.47 | 24.7 | 20.0 | 7.1 | 23.5 | 37.7 | 15.1 | .243 | .258 | .270 | .097 | .146 | .056 | | 1.13 | 1.08 | 1.04 | 51 | 8 | 12 | 15 | -- | 14 |
| 6 | 37 | 9/20/49 | | 1.03 | 0.22 | 0.79 | 15.3 | 9.3 | 8.0 | 14.8 | 42.3 | 10.1 | .261 | .245 | .278 | .057 | .172 | .036 | 0.89 | 0.28 | 0.25 | | UNSATISFACTORY | | | | | |
| | M | 9/29/49 | ACTH | 0.92 | 0.30 | 0.60 | 11.5 | 7.5 | 6.8 | 12.5 | 25.0 | 11.3 | .235 | .223 | .258 | .053 | .112 | .044 | | 0.50 | 0.43 | 0.43 | 30 | 7 | 15 | 22 | -- | 26 |
| | | 11/14/49 | ACTH | 0.79 | 0.30 | 0.46 | 14.0 | 8.1 | 6.6 | 17.7 | 27.0 | 14.4 | .244 | .237 | .277 | .073 | .116 | .052 | 1.63 | 0.64 | 0.55 | | UNSATISFACTORY | | | | | |
| 7 | 13<br>F | 2/1/50 | | 1.23 | 0.41 | 0.73 | 38.6 | 28.7 | 12.6 | 31.4 | 70.0 | 17.3 | .239 | .219 | .280 | .131 | .273 | .061 | 1.27 | 0.56 | 0.51 | 0.61 | 38 | 10 | 13 | 12 | 8 | 19 |

D* - OPTICAL DENSITY

-- NOT PRESENT

N.D. - NOT DONE

## TABLE VI

*Protein, sulfhydryl and electrophoretic data of sera from patients with miscellaneous diseases*

| 1 | 2 | 3 | 4 | | | 5 | | | 6 | | | 7 | | | 8 | | | 9 | | | | 10 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PAT. | AGE & SEX | DIAGNOSIS | TOTAL NITROGEN AS G./100 ml SERUM | | | μMOLES SH PER 100 ml SERUM | | | μMOLES SH PER G. OF NITROGEN | | | D~ PER mg NITROGEN | | | μMOLES SH PER D 100 ml SERUM | | | ALBUMIN / GLOBULIN | | | | PERCENT | | | | | |
| | | | SERUM | ALB. | GLOB. | SERUM | ALB. | GLOB. | SERUM | ALB. | GLOB. | SERUM | ALB. | GLOB. | SERUM | ALB. | GLOB. | HOWE | KJELD. | BIUR. | ELEC. | ALB. | α1 | α2 | β | M | γ |
| 1 | 66 F | RHEUMATOID ARTHRITIS 6/29/49 | 1.03 | 0.40 | 0.61 | 21.3 | 15.1 | 8.0 | 20.7 | 37.8 | 13.1 | .234 | .215 | .243 | .088 | .176 | .054 | 1.7 | 0.66 | 0.58 | 0.43 | 30 | 11 | 19 | 19 | -- | 21 |
| 2 | 53 M | GOUT 7/12/49 | 1.18 | 0.39 | 0.72 | 33.3 | 20.1 | 13.2 | 28.1 | 51.6 | 18.3 | .233 | .218 | .249 | .121 | .236 | .074 | | 0.53 | 0.47 | 0.59 | 37 | 6 | 11 | 18 | -- | 28 |
| 3 | 14 F | SUBACUTE GLOMERULAR NEPHRITIS 5/18/49 | 0.73 | 0.29 | 0.41 | 24.4 | 16.3 | 9.5 | 33.4 | 56.2 | 23.2 | .258 | .241 | .276 | .130 | .230 | .084 | | 0.71 | 0.63 | 0.52 | 34 | 9 | 26 | 19 | -- | 11 |
| 4 | 52 M | HEMOCHROMATOSIS CIRRHOSIS 5/17/49 | 1.00 | 0.28 | 0.69 | 20.9 | 11.4 | 10.1 | 20.9 | 40.7 | 14.6 | .246 | .218 | .258 | .085 | .187 | .057 | 0.89 | 0.40 | 0.34 | | N.D. | | | | | |
| 5 | 47 F | CIRRHOSIS CLONORCHIS SINENSIS 11/29/49 | 1.14 | 0.54 | 0.58 | 33.9 | 27.7 | 10.7 | 29.7 | 51.3 | 18.5 | .245 | .235 | .248 | .121 | .218 | .074 | 1.95 | 0.93 | 0.88 | | N.D. | | | | | |
| 6 | 33 F | HODGKIN'S DIS. 12/10/49 | 1.18 | 0.49 | 0.69 | 15.5 | 10.7 | 8.9 | 13.1 | 21.8 | 12.9 | .228 | .215 | .256 | .058 | .101 | .050 | | 0.71 | 0.60 | 0.45 | 31 | 16 | 19 | 16 | -- | 18 |
| | | ACTH 12/13/49 | 1.31 | 0.55 | 0.70 | 17.0 | 10.5 | 7.6 | 13.0 | 19.1 | 10.9 | .242 | .241 | .270 | .053 | .079 | .040 | | 0.79 | 0.71 | 0.52 | 34 | 13 | 15 | 19 | -- | 19 |
| 7 | 19 M | NEPHROTIC STAGE OF GLOMERULAR NEPHRITIS 6/6/50 | 0.49 | 0.08 | 0.41 | 10.6 | 2.6 | 9.0 | 21.5 | 31.3 | 22.2 | .251 | .265 | .267 | .085 | .118 | .083 | 0.50 | 0.20 | 0.20 | | SAMPLE UNSATISFACTORY | | | | | |
| 8 | 26 M | MONOCYTIC LEUKEMIA ACUTE 4/28/49 | 1.22 | 0.61 | 0.55 | 41.1 | 34.0 | 10.2 | 33.7 | 55.8 | 18.5 | .255 | .256 | .278 | .132 | .218 | .067 | | 1.11 | 1.02 | 0.72 | 42 | 10 | 15 | -- | 14 | 19 |
| 9 | 36 M | CYSTINURIA 10/6/50 | 1.22 | 0.62 | 0.55 | 58.0 | 43.1 | 18.0 | 47.5 | 69.5 | 32.7 | .225 | .237 | .239 | .211 | .293 | .137 | | 1.14 | 1.12 | 1.38 | 58 | 7 | 9 | 15 | -- | 11 |

D~ - OPTICAL DENSITY

N.D. - NOT DONE

-- NOT PRESENT

These totals do not reflect the source of the sulfhydryl value since the latter is a resultant of the albumin and globulin values. The only value for the albumin sulfhydryl which was in the normal range was that of patient 11. The further analysis of the albumin sulfhydryl with respect to micromoles of SH per gram of nitrogen or peptide unit (cols. 6 and 8; Figure 12) showed that there was a qualitative change in the composition of the serum albumin among many of these patients with malignancy. Most of the values were reduced, three were in the normal range, and one was above normal. When the peptide per mg nitrogen (col. 7) in this group was similarly examined, there was no distinctive abnormality noted even among those patients whose SH (expressed as micromoles per gram nitrogen [col. 6] or per peptide unit [col. 8]) was low. This implied that the protein, with respect to its nitrogen and peptide content, was not significantly different from the normal.

The globulin fraction of the serum as separated by the Pillemer fractionation was normal or increased in this group (col. 4). When the SH per gram of globulin nitrogen was calculated, sera 8b and 11 were high, samples 1, 4a, 7a and 9b approximated normal and the remainder were reduced in a varying degree from the normal (col. 6). When the SH was expressed per unit peptide, the sera of most patients showed a sub-normal ratio (col. 8). The sera from two patients (8b and 11) showed an increased ratio while in one patient (No. 5), the SH, although low when expressed per gram of nitrogen, was normal when expressed per unit peptide. With the exception of one markedly increased value (patient 4a), the ratio of peptide per mg of nitrogen (col. 7) in this group was within the normal range.

Electrophoretic analysis of these sera from patients with malignancy showed no apparent correlation between the sulfhydryl values and the distribution of the serum components. As has been noted in previous studies on sera from such individuals, the albumin component was uniformly reduced while the alpha globulins were usually elevated (16). Among normal individuals, the albumin-globulin ratios obtained by the Pillemer and electrophoretic procedures agreed. When these patients' sera were analyzed, discrepancies were found between the albumin-globulin ratios calculated on the basis of the Pillemer fractionation and the electrophoretic analysis. The differences between ratios determined by the Kjeldahl and the biuret method were small. Howe protein fractionation of these sera yielded albumin-globulin ratios which were all greater than 1.0 (1.5–2.0) with only one patient in the low range of 1.2 (Figures 1 and 2).

### *Multiple Myeloma*

Eight patients with diagnosis of multiple myeloma confirmed by bone marrow aspiration, biopsy and/or autopsy have been studied. Five were males and three were females. Their ages varied from 48 to 67 years. All had evidence of diffuse bone involvement on roentgen examination.

Detailed examination of the sera obtained from these patients is summarized in Table IV. The albumin nitrogen was subnormal in all patients while the globulin was markedly elevated in seven and normal in one. The sulfhydryl content of the serum was markedly reduced in six patients, normal in one, and above normal in another patient (col. 5). The micromoles of albumin sulfhydryl were reduced far below normal in all and nondetectable in one case (col. 5). The globulin SH varied from subnormal to markedly increased values. The micromoles of SH per gram of protein nitrogen (col. 6) were low in the sera of all patients. With a single exception (patient 2), the same was true of the albumin SH per gram of nitrogen. The SH per gram of globulin nitrogen was reduced in six and above normal in two instances (Nos. 2 and 5). These same relationships were noted for the SH per unit of albumin and globulin peptide (col. 8). No characteristic change in the two sera with high SH values was observed when the peptide per mg of nitrogen of the albumin and globulin components was examined (col. 7).

One serum (No. 2), with a high SH per gram nitrogen or per peptide unit in both albumin and globulin fraction, appeared to differ on this determination from the other cases in this group. Its electrophoretic pattern was characterized by an extremely low albumin content and a globulin fraction which contained 22% of the total protein as M protein and 57% as gamma globulin. However, this electrophoretic distribution was not markedly different from that of other sera in this series, the sulfhydryl values of which were low.

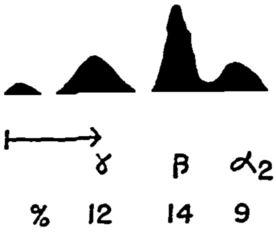

FIG. 1. NORMAL ELECTROPHORETIC PATTERN OBTAINED WITH THE APPARATUS AND METHOD DESCRIBED
(Current—15.2 milliamps., time—9,960 seconds)

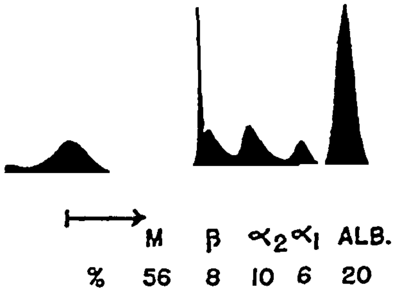

FIG. 3. PATIENT 1, TABLE IV, SERUM 12/8/49—MULTIPLE MYELOMA WITH M PROTEIN
(Current—15.1 milliamps., time—10,800 seconds)

It is of interest to note that of seven patients whose sera were analyzed electrophoretically, the sera of four showed the presence of M protein. The serum of one of these four patients contained no gamma globulin. The sera of six of the seven patients showed a high proportion of either M protein or gamma globulin. The serum of patient 6, which did not show a high content of M protein or gamma globulin, was found to have sulfhydryl values for albumin and globulin which were in the low range (Figures 3–5).

The ratio of albumin to globulin was low in the

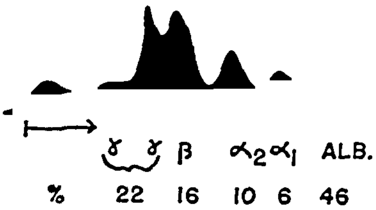

FIG. 2. PATIENT 11, TABLE III—MELANOCARCINOMA WITH ELEVATED SULFHYDRYL VALUES IN THE PROTEIN COMPONENTS
(Current—15.1 milliamps., time—10,800 seconds)

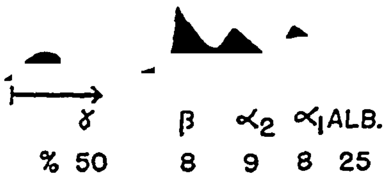

FIG. 4. PATIENT 3, TABLE IV, SERUM 3/3/50—MULTIPLE MYELOMA WITH INCREASED GAMMA COMPONENT
Note similarity to that of 3/6/50 in Table IV (not illustrated). (Current—15.0 milliamps., time—9,000 seconds)

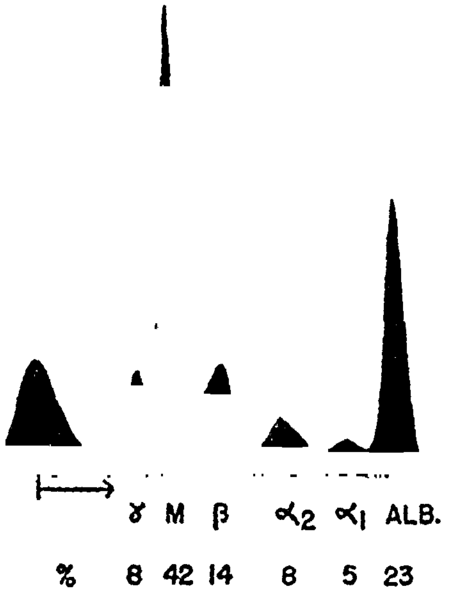

Fig. 5. Patient 5, Table IV, Serum 3/15/50—Multiple Myeloma with Both M and Gamma Components (Current—15.1 milliamps., time—10,800 seconds)

sera from all patients by all four methods of comparison. The only possible exception was the serum of patient 6 in which the ratio with the Howe method was normal. Its electrophoretic pattern showed a reduction in the gamma globulin, absence of M protein and high $\alpha_1$ and $\alpha_2$ globulin components.

Patient 2, with multiple myeloma, had a localized tumor in a vertebra which was removed free of bone or other tissue at autopsy and frozen immediately. After this tumor had been ground in a Waring Blendor at 0°C, and extracted with 0.14 M saline, the suspension was clarified by centrifugation at 13,000 rpm at 0°C and filtered through a coarse sintered filter. Analysis of this extract showed a nitrogen content of 1.5 mg per ml. The sulfhydryl content of this extract was .19 micromole per ml or 127 micromoles of SH per gram nitrogen. The SH per peptide unit was 0.328 micromole. This latter value equaled the value 0.331 for micromoles of SH found in the patient's serum albumin. Electrophoretic analysis of this tumor extract showed a pattern of peaks which resembled somewhat the serum pattern in distribution and mobility - albumin 14%, alpha$_1$ 16%, alpha$_2$ 9%, M protein 4.0% and gamma globulin 57%. The high SH per gram of nitrogen observed with this extract as well as the increase in the ratio of peptide to total nitrogen (0.387) probably reflects the presence of intracellular protein components whose amino acid composition differed from the proteins observed in serum.

### *Lupus Erythematosus*

Seven patients diagnosed as suffering from lupus erythematosus disseminata were selected for study because of the abnormality frequently observed in the serum proteins in this disease, and our previous experience which had indicated that the total serum sulfhydryl content was markedly reduced in this condition. The cases selected for further study had shown clinical and laboratory observations characteristic of the disease. Five patients were females and two were males. Their ages ranged from 16 to 37. The observations on these patients with lupus erythematosus are summarized in Table V. The serum proteins were altered with a low albumin and normal or high globulin content in all instances (col. 5). The total serum sulfhydryl was below normal in all patients including those specimens obtained while the patient was receiving ACTH (col. 6). The albumin sulfhydryl per 100 ml serum was low in all sera (col. 6) and the serum of only one patient (No. 7) had a normal sulfhydryl per gram of albumin nitrogen (col. 7). The globulin sulfhydryl per 100 ml of serum varied from normal to subnormal and none were normal when expressed per gram globulin nitrogen (col. 7). The same relationships were observed when the sulfhydryl analysis per peptide unit was calculated for both the albumin and globulin components. The peptide to nitrogen ratio of the total serum proteins and albumin did not show any singular deviation although this ratio for the globulins was distinctly skewed toward a higher value (col. 8). As may be noted, although albumin-globulin ratios were diminished, the extent varied with the technique employed (col. 10).

### *Miscellaneous Disease*

A group of patients was observed with miscellaneous diseases, in many of which abnormalities in serum proteins have been noted. The clinical entities, ages and observations on this group are summarized in Table VI. The albumin nitrogen was reduced in the sera of all these patients while the globulin nitrogen was normal or increased. The amount of albumin present (col. 4) and the sulfhydryl detected in this fraction (col. 5) in the serum of patient 7, was so low that the derived ratio is of questionable significance. With the exception of the cystinuric patient (No. 9), the sulfhydryl content per 100 ml serum in this group was below the normal. This was also true of the SH in the albumin fraction. No consistent change, except for the high value for patient 9, was noted in the globulin sulfhydryl (col. 5). The sulfhydryl of the globulin, per peptide unit, in the sera of the two nephrotic patients approximated the normal value and that for the cystinuric patient was above the normal range (col. 8). The peptide content per unit nitrogen in the serum proteins showed no consistent deviation from the normal (col. 7). The sulfhydryl per peptide unit was below normal for all the albumin and globulin samples, with the exception of the patient with cystinuria. The heterogeneity of this group of patients does not permit generalization of these data.

### EFFECT OF THERAPY

The effect of various therapeutic measures on the serum sulfhydryl value could be observed among several patients in these disease groups. One patient (No. 1, Table V) received 125 grams of intravenous salt free human albumin[2] during the period between the two serum samples which were examined. The albumin nitrogen of the serum rose from 0.27 to 0.37 gram per 100 ml and the globulin fell from 0.80 to 0.73 gram of nitrogen per 100 ml of serum. The sulfhydryl content of the albumin and globulin fractions rose from 14.7 to 18.9 and 12.3 to 13.4 micromoles of SH per 100 ml of serum respectively. However, the sulfhydryl for albumin decreased from 54.4 to 51.1 micromoles SH per gram nitrogen and from 0.223 to 0.191 micromole SH per peptide unit. The SH per unit globulin nitrogen and per unit globulin peptide rose during this period although the globulin content decreased. The total serum protein nitrogen remained unchanged but the components shifted as indicated by the albumin- globulin ratios before and after albumin therapy. These observations are of interest because the albumin administered contained only 15.7 micromoles SH per gram of nitrogen instead of the normal 63.6 micromoles.

Serum was obtained from four patients receiving ACTH, prior to and during the period of therapy. The data are presented in Table V (patients 4–6) and Table VI (No. 6). The first patient (Table V, No. 4) was a 16 year old female suffering from acute disseminated lupus. She had received 900 mg of ACTH (Armour), over a period of 11 days, with objective clinical improvement. Her serum showed a rise in the albumin component with a decrease in the gamma globulin and disappearance of the M component. A fraction, with the mobility of $\beta$ globulin, which had previously been absent, appeared. The total sulfhydryl of the serum rose, and, whereas the albumin sulfhydryl had been zero, the subsequent determination showed 37.9 micromoles of SH per gram of albumin nitrogen. During the next two week period, she received an additional 677 mg of ACTH. Serum obtained one month later showed progressive changes toward a more normal protein distribution electrophoretically. The sulfhydryl content of the albumin and globulin fractions per unit nitrogen and per unit peptide rose but did not attain the normal value. The serum showed a total protein content within the normal range as a result of the reduction in the globulin content and increase in the albumin. The albumin-globulin ratio was still markedly abnormal (Figures 6 and 7).

The second patient (Table V, No. 5) was diagnosed as having lupus erythematosus disseminata. He had received 480 mg of ACTH in daily amounts of 40 to 100 mg for the first seven days. ACTH therapy was continued with progressively smaller daily doses of 36, 21, 15, and 9 mg during the ensuing month. Sera were obtained for study five days after the ACTH therapy was instituted and a month later while he was in remission and receiving maintenance doses of the hormone. In this period, the total protein nitrogen had increased from 0.88 gram to 1.05 grams per 100 ml serum. The albumin fraction which was 0.44 gram initially rose to 0.53 gram of nitrogen per 100 ml and the globulin which had been 0.42 gram of nitrogen per 100 ml of serum was 0.47 gram on the later specimen. The total sulfhydryl of the serum proteins decreased somewhat due to a slight fall in both the albumin and globulin content. The fall in SH was even more apparent in the albumin and globulin SH components when the SH per gram nitrogen or per peptide unit was measured (cols. 7 and 9, Table V). Despite the improvement in the albumin content of the serum and in the electrophoretic analysis, the sulfhydryl values were lower and did not parallel the changes in the protein values.

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[2] We have examined a similar preparation of human serum albumin for parenteral use (without preservative and salt poor). It was prepared by E. R. Squibb and Co., from plasma collected by the Red Cross (control No. 3024–2). The solution was clear and was stated to contain 25% albumin. Our studies showed that it contained 42 mg nitrogen per ml and the optical density by the biuret method was 9.83 per ml. The SH values were 15.7 micromoles per gram of nitrogen. The D per mg of nitrogen was .234 and the SH per D was .067.

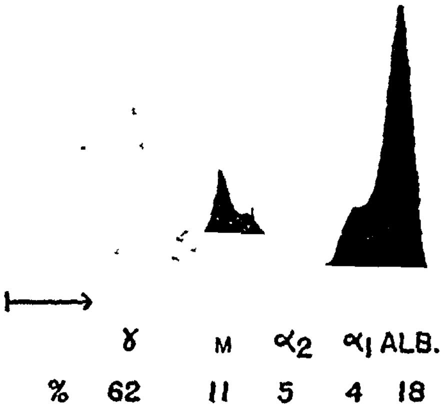


Fig. 6. Patient 4, Table V, Serum 7/30/49—Lupus Erythematosus Before ACTH Administration

No albumin sulfhydryl was detected. (Current—15.1 milliamps., time—9,660 seconds)

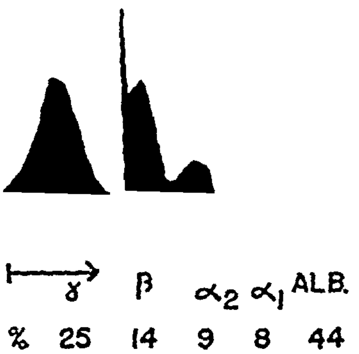


Fig. 7. Patient 4, Table V, Serum 9/20/49—Patient Had Received ACTH

Serum albumin sulfhydryl approximately normal. (Current—15.1 milliamps., time—7,200 seconds)

A 37 year old male suffering from acute disseminated lupus (Table V, No. 6) received 100 mg of ACTH daily for eight days and thence 985 mg in varying daily dosage for the following 38 days. He developed a bacteremia during this period and staphylococci of both aureus and albus types were isolated from his blood. Two to 3 grams of aureomycin per day were administered for the first two weeks and for the following two months he received 4.5 grams of aureomycin daily. Specimens of serum from this patient were obtained just preceding and nine days after the initiation of ACTH therapy. Another specimen was obtained during the period that both aureomycin and ACTH were being administered. During the first period while he was receiving ACTH, a fall in the total serum protein was observed with a slight increase in the albumin content and decrease in the globulin. The sulfhydryl in the albumin decreased and that in the globulin increased per gram of nitrogen or per peptide unit (cols. 7 and 9). During the following period when aureomycin was administered together with the ACTH because of the bacteremia, the albumin remained unchanged at its low level, but the globulin nitrogen decreased to the normal level. The albumin sulfhydryl remained unchanged while the globulin sulfhydryl per gram of nitrogen continued to increase. It may be noted that the ratio of albumin to globulin was persistently abnormal in this patient's sera.

The fourth patient (Table VI, No. 6) was a 33 year old female with Hodgkin's disease. She had received 300 mg of ACTH over a period of three days. During this period a dramatic improvement in her clinical status had occurred. The patient became afebrile within 24 hours and had a diuresis with the loss of 13 lbs. in body weight. No essential change in the protein or sulfhydryl content of the serum occurred. Ten other patients with lupus, rheumatic fever or rheumatoid arthritis were treated with ACTH for short periods of time. The total serum sulfhydryl as well as serum protein determinations were performed on samples obtained before and during this therapy. In general, a slight decrease in the serum protein nitrogen was observed with a concomitant small increase of sulfhydryl per gram of nitrogen. These unfractionated samples cannot be critically evaluated and the changes observed probably represent a slight increase in the albumin fraction with a decrease in the globulin content of the serum.

Among the patients with malignant tumors (Table III) there were several from whom serial specimens of serum were obtained while they had been receiving folic acid antagonist therapy, either Aminopterin or A-Methopterin. Patients 1, 2, 4, 6, and 7 had shown no objective or apparent clinical response to the therapy. One patient (No. 3) showed local regression of an ulcerated carcinoma of the breast. Two patients (Nos. 8 and 9) manifested temporary regression of the metastatic lesions and clinical improvement. The latter two patients showed a definite change in the sulfhydryl of the serum proteins during this period. Patient 8 had pleural metastases from a carcinoma of the breast with recurrent sanguinous effusions in which carcinoma cells had been observed. She received 5 to 15 mg of A-Methopterin daily, and, after three weeks, x-ray examination of the chest showed almost complete clearing of the pleural fluid. Reaccumulation of the fluid was not observed during the ensuing four months of observation. She gained weight and was asymptomatic. From the time just preceding A-Methopterin therapy to the time one month later when A-Methopterin was discontinued prior to discharge from the hospital, the total protein in the serum had decreased with a diminution of both albumin and globulin components. The sulfhydryl per gram albumin or globulin nitrogen more than doubled during the interval. The micromoles of sulfhydryl per peptide unit increased from .135 to .214 for the albumin fraction and from .051 to .117 for the globulin. The second patient (No. 9) had a rhabdomyosarcoma of the left leg with pulmonary metastases. Amputation of the leg had been performed one year previous to this hospital admission. He had lost 20 lbs. in weight, had a paroxysmal cough and drenching night sweats from January through April, 1950. A-Methopterin, 10 mg daily, was administered and within two weeks distinct clinical improvement was noted which was progressive so that the patient left the hospital and felt well enough to attend to his business. X-ray examination of the chest had shown disappearance of some of the pulmonary shadows and diminution in the size of other shadows. The patient had gained 14 lbs. in weight. A-Methopterin had been reduced to 5.0 mg orally per day. One month later, an increase in the size of the pulmonary metastases was noted by roentgen examination with subsequent return of symptoms. These were progressive despite increase in dosage of A-Methopterin to 10 mg per day so that he was rehospitalized. Sera obtained before and during this period of folic acid antagonist therapy showed changes in the sulfhydryl content which paralleled the clinical observations to some extent. Preceding the folic acid antagonist therapy, an increased globulin and low albumin content of the serum was present. The sulfhydryl content of these fractions was low. During the period of clinical improvement, the albumin nitrogen of the serum increased with no apparent change in the globulin. The sulfhydryl increased in both albumin and globulin fractions, to the normal range, when expressed either as micromoles per gram of nitrogen or per peptide unit. With clinical relapse, although still receiving A-Methopterin, the albumin nitrogen of the serum decreased, but the globulin appeared to remain unchanged. The sulfhydryl, per gram albumin nitrogen or peptide unit, decreased rapidly to the original pre-therapy level. The globulin sulfhydryl also decreased. Electrophoresis of these serum samples showed that a decrease in the albumin component occurred during this relapse with an increase in the alpha globulin. The folic acid antagonists do not appear to affect the globulin fraction of the serum in a manner comparable to the uniform decrease observed with ACTH (Figures 8 and 9).

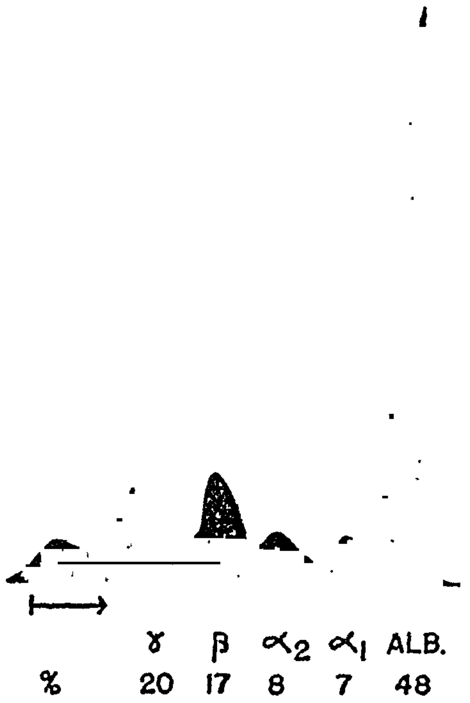


FIG. 8. PATIENT 9, TABLE III—SERUM SAMPLE OBTAINED ON 6/8/50 WHEN PATIENT WAS IN A CLINICAL REMISSION WHILE RECEIVING A-METHOPTERIN
(Current—15.2 milliamps., time—10,800 seconds)

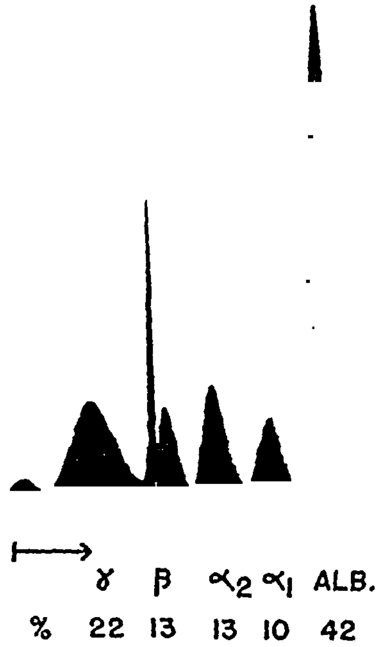


FIG. 9. SAME PATIENT AS FIGURE 8—SERUM SAMPLE OBTAINED ON 7/31/50 DURING CLINICAL RELAPSE
(Current—15.1 milliamps., time—9,000 seconds)

## DISCUSSION

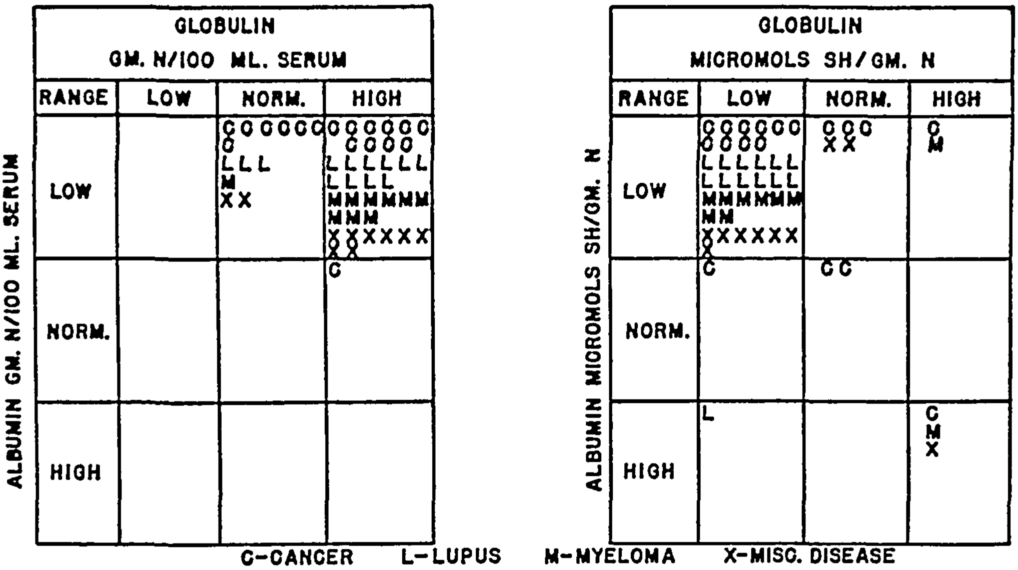
Kolthoff and Lingane have summarized the results of polarographic studies of blood, based on the presence or absence of sulfhydryl groups (17). Black (18) has reported alterations from the normal in the sulfhydryl content of plasma and serum, *from patients with neoplastic disease*, as measured by the rate of reduction of methylene blue and other dyes. Glass (19) and Huggins and his colleagues (20) have studied the coagulation of the serum proteins as an index of their albumin content. They have also presented observations indicating that when an alkylating agent such as iodoacetic acid is added to serum, presumably reacting with sulfhydryl groups, the results confirmed the defect noted in various pathologic states when simple coagulation was employed (21).

From the observations reported in the present paper, it is apparent that a reduction in the total sulfhydryl in the serum proteins frequently occurs in various diseased states. Fractionation of the serum and determination of the sulfhydryl in each fraction showed that the total value for the serum protein sulfhydryl was the resultant of those obtained for the albumin and globulin fractions. The sera of normal individuals contain approximately two and one half times as much SH per gram of albumin nitrogen as per gram globulin nitrogen (13). A reduction in the albumin nitrogen will therefore usually result in a lowered total serum protein sulfhydryl even when an increase in the globulin component occurs. In the majority of the patients studied, the sulfhydryl per gram of albumin or globulin nitrogen in the sera was reduced, and despite marked elevation of the globulin content in various diseases, the total SH of the serum was rarely normal or above the normal.

The deviations from the normal in the sera are graphically presented in Figures 10–12.

As previously noted (see Methods), the qualitative nature of a protein may be established by several indices. One index would be the number of peptide bonds per mg of nitrogen. This is a function of the types of amino acids present in the protein. In Figure 11, the results of such an analysis are presented. It will be noted that the results with the sera from these patients show a distribution about the normal. A second index that may be employed is the relationship between the number of sulfhydryl groups per unit of protein nitrogen or peptide $\left(\frac{\mathrm{SH}}{\mathrm{nitrogen}} \text{ or } \frac{\mathrm{SH}}{\mathrm{D}}\right)$. These values characterize the protein with respect to a specific type

RELATIONSHIP OF ALBUMIN TO GLOBULIN NITROGEN AND SH VALUES AMONG PATIENTS WITH DISEASE

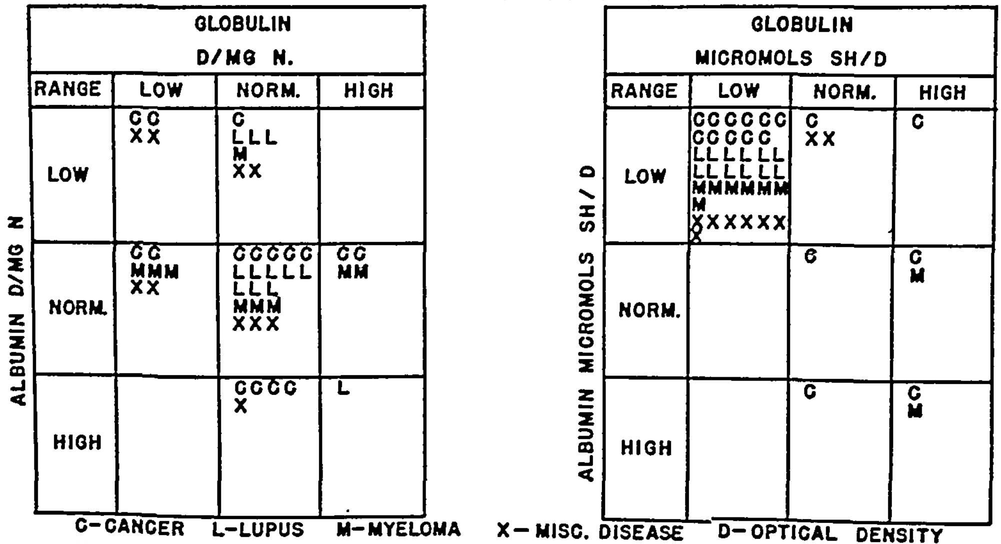


C–CANCER L–LUPUS M–MYELOMA X–MISC. DISEASE

FIG. 10. DISTRIBUTION OF ALBUMIN AND GLOBULIN NITROGEN AND ALBUMIN AND GLOBULIN SH PER GRAM PROTEIN NITROGEN IN PATIENTS WITH DISEASE

of linkage other than the peptide bond common to proteins and polypeptides. In Figure 10, the SH per gram of albumin and globulin nitrogen is represented. Eighty-six % of the albumin values and 76% of the globulin values were below the normal range. The SH per peptide unit, presented in Figure 11, shows that 87% of the albumin and 79% of the globulin determinations were subnormal. Seventy-five % of all the sera examined from these patients was subnormal in both albu-

RELATIONSHIP OF ALBUMIN TO GLOBULIN NITROGEN, PEPTIDE AND SH VALUES AMONG PATIENTS WITH DISEASE



C–CANCER L–LUPUS M–MYELOMA X–MISC. DISEASE D–OPTICAL DENSITY WITH BIURET.

FIG. 11. PEPTIDE PER MG NITROGEN IN THE ALBUMIN AND GLOBULIN COMPONENTS AND THE SH PER PEPTIDE UNIT FOR THESE FRACTIONS AMONG PATIENTS WITH DISEASE

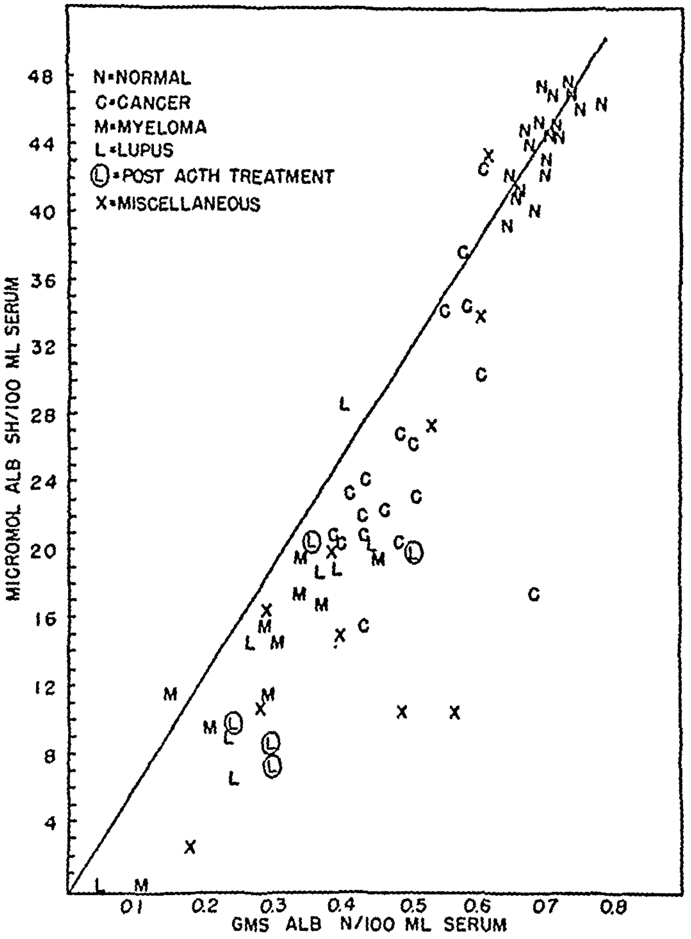


FIG. 12. DISTRIBUTION OF THE ALBUMIN SH PER GRAM OF NITROGEN AMONG PATIENTS WITH DISEASE

min and globulin SH values whether expressed as SH per gram of nitrogen or per peptide unit. The skewness of these observations is in marked contrast to those for the peptide per nitrogen index described previously $\left(\frac{D}{\text{mg nitrogen}}\right)$. The albumin fraction in these sera was low both in terms of protein nitrogen and sulfhydryl. The relationship between the albumin SH values and the albumin nitrogen content is graphically presented in Figure 12. It will be noted that the SH in the albumin fraction was reduced below the normal range in all sera except for one patient with a melanocarcinoma (Table III, No. 11) and one patient with cystinuria (Table VI, No. 9). The ratio of sulfhydryl to nitrogen was generally reduced below that obtained with normal sera.

These observations indicate that in diseased states, irrespective of the increase or decrease in protein content, a qualitative change occurs in the sulfhydryl content of the serum protein components. The changes in both albumin and globulin sulfhydryl are in the same direction and may be *the manifestation of a similar alteration in* protein synthesis or rearrangement of molecular architecture. No distinctive abnormality, characteristic for the different disease processes, was apparent.

Further study of tissue components and serum fractions is being conducted in the hope that the mechanism responsible for the metabolic abnormality which results in the altered protein sulfhydryl observed in various disease states may be described.

## SUMMARY

1. The sera from patients with malignant tumors, lupus erythematosus, multiple myeloma, and miscellaneous diseases associated with abnormality of the serum proteins have been investigated with respect to the albumin and globulin content as measured by the protein nitrogen, biuret method and electrophoresis.

2. The sulfhydryl content of the serum protein as well as of the albumin and globulin components has been determined employing an amperometric *titration technique*.

3. A reduction in the albumin component with normal or increased globulin has been observed in these sera but the peptide per mg of nitrogen did not deviate markedly from the normal.

4. The sulfhydryl per mg of protein nitrogen or per peptide bond was subnormal in both the albumin and globulin components in 75% of these sera.

5. It is believed that a metabolic abnormality related to protein formation exists in these disease states which is reflected in the low sulfhydryl values in the albumin and globulin fractions.

6. The changes in the serum protein and the sulfhydryl values in patients who received ACTH or folic acid antagonist therapy are presented. The number was too small for generalization with respect to the mechanism of action of these agents.

## ACKNOWLEDGMENTS

We wish to thank Drs. A. McGehee Harvey, John E. Howard, Sherman M. Mellinkoff, Francis P. Chinard, Ezra M. Greenspan, Jacob Colsky, Stanton Eversole, and B. Abeshouse for their cooperation and aid in these investigations.

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# AN ESTIMATION OF THE RENAL AND EXTRARENAL CLEARANCE OF RADIOIODIDE IN MAN

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(Submitted for publication February 19, 1951; accepted April 23, 1951)

The growing importance of radioiodine to clinical practice and medical research has stimulated interest in various kinetic aspects of iodine metabolism, including the renal excretion of iodide as estimated by the renal clearance of iodide (1–3). In addition, it has been suggested that the most explicit and precise measure of the iodine-accumulating function of the thyroid gland may be the clearance of iodide by the thyroid (3, 4), defined by analogy as the volume of plasma or serum cleared by the thyroid gland of its iodide content per minute, and determined from multiple observations *in vivo* of the thyroid itself compared with observations of serum. Since most of the circulating iodide eventually reaches the urine or the thyroid gland, it has also been observed (1) that a useful indirect measure of clearance of iodide by the thyroid called "extrarenal iodide clearance," may be obtained from observations on serum plus urinary excretion alone, without recourse to observations *in vivo*.

Although the foregoing concepts and methods of estimation have been discussed at length, few actual values for such clearances have been published. It is the purpose of this report to present values for renal and extrarenal clearance of radioiodide in a group of patients illustrating various states of thyroid and renal function.

## METHODS

After the administration by mouth of 100 microcurie tracer doses of radioiodine, along with 100 micrograms of sodium iodide as carrier, samples of blood serum and of urine, collected at suitable intervals, were analyzed for their content of $I^{131}$. The methods employed for collections and analyses have been described elsewhere (1, 5). Clearances were calculated by the method described by Berkson and associates (6). The renal clearance of iodide was obtained by use of the formula $Cl = \frac{U'_t}{P'_0} r$, in which $U'_t$ is the estimated asymptotic value of the renal excretion curve expressed as a fraction of the dose administered and r the exponential rate constant of that curve, and $P'_0$ is the concentration of inorganic $I^{131}$ in the blood at zero time obtained by extrapolation. Extrarenal clearance of iodide was calculated by the use of the formula $\frac{r(1 - U'_t)}{P'_0}$, and is the difference between total clearance and renal clearance.

## RESULTS

Renal clearances of iodide have been estimated for 43 individuals by the foregoing method. Fifteen of the patients studied had exophthalmic goiter, one had adenomatous goiter with hyperthyroidism, six had myxedema and 21 were euthyroid (15 with normal thyroids, two with carcinoma of the thyroid and four with adenomatous goiter without hyperthyroidism).

Twelve of the 15 patients with normal thyroid glands who were studied had severe renal insufficiency, and had been hospitalized for treatment of this condition. The various diagnoses of the renal disease among these 12 patients were glomerulonephritis, nephrosis, myeloma, cardiac decompensation and intracapillary glomerulosclerosis.

Although tests of renal function were not performed in the other 31 patients, none of them made any complaint referable to the kidneys and studies or laboratory tests were not indicative of serious renal disease. It was assumed that their renal function was normal, or at least nearly so.

Table I gives the mean values for renal clearance of iodide among the patients studied.

TABLE I

*Renal iodide clearance in cubic centimeters of serum cleared per minute*

| Condition | Renal function* | | | |
|---|---|---|---|---|
| | Presumably normal | | Definitely impaired | |
| | Cases | Iodide clearance | Cases | Iodide clearance |
| Hyperthyroidism | 16 | 32.7 ± 1.0 | | |
| Euthyroidism | 9 | 33.3 ± 3.1 | 12 | 8.7 ± 1.6 |
| Myxedema | 5 | 17.9 ± 1.6 | | |

* The value following the ± is the standard error of the mean.

In Table II are listed the extrarenal clearances of iodide obtained. For patients who had hyperthyroidism, the mean value was 154.6 cc. of serum cleared of radioiodine per minute; for euthyroid persons with presumably normal renal function it was 26.3 cc. per minute; for euthyroid patients with impaired renal function it was 6.5 cc. per minute; and for patients with myxedema it was 4.7 cc. per minute. It should be noted that there is very little overlapping of one group by another.

## COMMENT

The values for renal clearance of iodide reported here compare closely with those found by Myant and his associates (3), who, in a study in which radioiodine also was used, observed mean clearance rates of 31 cc. per minute among 11 euthyroid persons and 27 cc. per minute among 11 persons with untreated thyrotoxicosis. In studies in which large amounts of nonradioactive sodium iodide were administered, Elsom and his co-workers (7) found that the kidney of the dog cleared 9.0 cc. of plasma per minute of its iodide content, and Nelson and his associates (8) found the range of clearance of renal iodide in 13 normal men to be between 10 and 25 cc. per minute. It is of interest that the clearance of iodide in physiologic amounts (as observed in the studies in which radioiodine was used) was greater than the clearance estimated when much larger quantities of iodide were given to human beings and to dogs.

In so far as the clearance of tracer amounts of $I^{131}$ may be taken as a valid measure of the clearance of the nonradioactive inorganic iodide of the plasma, the clearance of the latter would appear to be greater than that of other halogens, such as chloride or bromide.

TABLE II

*Extrarenal clearance of iodide: 43 cases*

| Condition | Cases | Serum cleared per minute, cc. | |
|---|---|---|---|
| | | Range | Mean* |
| Hyperthyroidism | 16 | 49–479 | 154.6 ± 19.5 |
| Euthyroidism with presumably normal renal function | 9 | 10–64 | 26.3 ± 5.2 |
| Euthyroidism with impaired renal function | 12 | 3–11 | 6.5 ± 0.2 |
| Myxedema | 6 | 3–8 | 4.7 ± 0.7 |

* The value following the ± is the standard error of the mean.

The values for extrarenal clearance of iodide found in this study correspond to the thyroid clearance rates reported by Myant and his associates (3) and by Stanley (9). The former investigators found the mean thyroid clearance in 11 normal persons to be 16.0 cc. per minute and in 11 thyrotoxic patients, 486 cc. per minute. Stanley found the thyroid clearance of iodide in three normal persons to be 18.6, 7.2 and 28.7 ml. per minute. Extrarenal clearance in euthyroid and hyperthyroid persons is a close approximation of thyroid clearance but in myxedematous persons this is not so.

Extrarenal clearance was consistently lower among the patients who had impaired renal function. This inferred reduction in thyroidal iodide clearance may not necessarily imply that the thyroid glands in this group accumulated less iodine per unit of time, since the average concentration of inorganic iodide in plasma may well be higher in these cases.

## SUMMARY

Doses of $I^{131}$ were administered to 43 persons, and from samples of urine and serum collected at suitable intervals, renal clearances of $I^{131}$ were calculated by a method described by Berkson and associates (6). Mean clearances in the individuals presumed to have normal renal function were 32.7 cc. per minute for persons who had hyperthyroidism, 33.3 cc. per minute for euthyroid persons, and 17.9 cc. per minute in patients who had myxedema. The clearances among persons who had impaired renal function were markedly decreased.

A method is also given for the determination of the extrarenal clearance of radioiodide (which is an approximation of thyroid clearance of iodide in all except hypothyroid persons). The hyperthyroid patients studied had a mean extrarenal clearance of iodide of 154.4 cc. per minute; the euthyroid persons with presumably normal function, 26.3 cc. per minute; the euthyroid persons with impaired renal function, 6.5 cc. per minute; and persons with myxedema, 4.7 cc. per minute.

Little overlap was found between any two groups. This clearance rate appears to be a sensitive index of thyroid function.

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# THE PURPLE COLOR REACTION GIVEN BY DIPHENYLAMINE REAGENT. I. WITH NORMAL AND RHEUMATIC FEVER SERA[1]

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(Submitted for publication February 2, 1951; accepted May 7, 1951)

Niazi and State (1) recently reported that when human serum was heated with the diphenylamine reagent of Dische (2, 3) a purple color developed. They also observed that in certain disease states, *e.g.*, carcinoma, pulmonary tuberculosis and rheumatic fever, the purple color reaction was of considerably greater intensity than in the serum of normal persons. We observed this color reaction when testing saline extracts of bovine tonsils for the presence of desoxyribonucleic acids using the Dische diphenylamine reagent. Subsequent fractionation of the tonsil extracts revealed that the substance which gave the purple color reaction with diphenylamine was a mucoprotein with an unusually low isoelectric point ($pI_e < 2.0$) (4). In confirmation of certain findings of Niazi and State (1) we have observed that serum from rheumatic fever and rheumatoid arthritis patients gives a color of greater intensity than does the serum from normal individuals.

The resulting purple solution has one absorption band in the visible spectrum, with a maximum at 530 mμ. This same absorption band is present when either serum or our purified mucoprotein from beef tonsils (4) is tested.

In this report we shall present observations with respect to the reaction, and suggest modifications in technique. We have applied the method to rheumatic fever sera, the results of which are included.

## *Conditions of the reaction*

The addition of Dische's reagent[3] directly to serum produces upon heating a turbid colored solution unsuitable for colorimetric readings. When serum proteins are precipitated with 20% trichloroacetic acid solution, the purple color reaction is present in the acid hydrolyzate obtained from the precipitate and absent in the filtrate. The moiety giving the purple color is dialyzable from serum only after acid or alkaline hydrolysis. In the procedure described by Niazi and State (1), the proteins are precipitated from serum with a 20% trichloroacetic acid solution, the supernatant discarded, the precipitate hydrolyzed with a 5% trichloroacetic acid solution, and the clear supernatant obtained after centrifugation is tested with the diphenylamine reagent. Since the 20% trichloroacetic acid filtrate from serum does not contain any interfering substance we have found it satisfactory to hydrolyze the serum directly. A study of the hydrolysis step shows that the intensity of the purple color reaction increases with time up to 20 minutes and then decreases slowly and becomes negative after several hours. For this reason we have adopted 20 minutes as a standard time of hydrolysis.

The time of heating the serum hydrolyzate and the reagent is another important factor in the intensity of the color produced. A plot of color intensity (in terms of optical density at 530 mμ) against time of heating shows a rapid increase with time up to two hours, after which the rate of increase is slight. When the heating is continued, the purple color shifts to blue (clearly noticeable at two hours) and the absorption curve develops a new band with a maximum at 650 mμ in addition to the one at 530 mμ. The variation in the shape of the absorption curves with time of heating is presented in Figure 1. We have selected 30 minutes as a standard time of heating; at this time an intense purple color is obtained with little interference from the blue band, as can be seen in Figure 1.

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[1] Supported in part by grants from the United States Public Health Service and from the Chicago Heart Association.

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[3] Composition of the reagent: glacial acetic acid 100 ml., conc. sulfuric acid 2.75 ml., diphenylamine 1 gm.

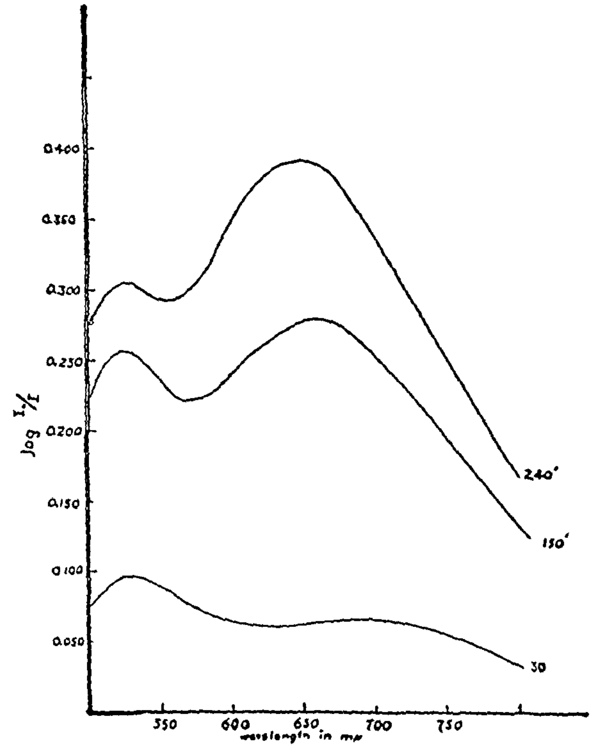

FIG. 1. VARIATION IN THE ABSORPTION SPECTRUM WITH THE TIME OF HEATING THE REACTION

Curves are shown for 30, 150, and 240 minutes of heating in a boiling water bath. Absorption measurements were made on a Model DU Beckman Spectrophotometer.

Varying the concentration of diphenylamine in the reagent between 0.5 and 1.5% has little effect upon the color development. We have maintained the 1% concentration of the original Dische reagent. On the other hand, the sulfuric acid concentration of the reagent has a marked influence on the intensity of the color, the intensity increasing as the acid concentration increases up to 10% in volume, where the curve becomes asymptotic. Therefore, replacement of the 2.75 ml. of concentrated sulfuric acid used in the original Dische formula with 10 ml. is useful in detecting the moiety when it is present in low concentrations. In this report we shall call the reagent containing 10 ml. of concentrated sulfuric acid "sensitive reagent",[4] and the one prepared according to Dische's formula "standard reagent."

It is important to note that the absorption curves obtained from serum using either standard or sensitive reagent have the same general shape provided a standard time of heating of 30 minutes is employed in both cases. This can be seen in Figure 2.

Using a Model DU Beckman Spectrophotometer we have determined that the color reaction obeys Beer's Law over the range of optical densities with which we have worked. Measurements included in Table I and in Figure 3 were made with a Model 6A Coleman Spectrophotometer. With this instrument when the optical density measured was greater than 0.650 it was customary to dilute the sample and multiply the reading obtained with the diluted sample by the appropriate dilution factor.

### *Suggested procedures*

On the basis of these findings, the following procedures are recommended:

*a) Macro procedure:* Two ml. of the sample (plasma or serum), are placed in a 25 × 150 mm. test tube, and 10 ml. of a 5% trichloroacetic acid solution are added slowly with gentle shaking in order to obtain fine floccules of precipitate. The tube is then placed in a boiling water bath for exactly 20 minutes and immediately cooled by immersion in ice water. The tubes are stoppered during the hydrolysis with a perforated rubber stopper fitted with 20 cm. long glass tubing which acts as a condenser. The hydrolyzate is filtered, and 1.5 ml. of the clear filtrate is transferred to a 18 × 150 mm. o.d. test tube to which are added 3 ml. of standard reagent. The tube is then immersed in a boiling water bath for exactly 30 minutes. After removal from the boiling water the tube is cooled in ice water and allowed to come to room temperature. The color intensity is stable for several hours at room temperature. The optical density was read in a Model 6A Coleman Spectrophotometer at 530 mμ in the 18 × 150 mm. test tube in which the color was developed. A large number of 18 × 150 mm. Pyrex test tubes were examined for uniform optical properties. Tubes were selected which were uniform to ± 0.005 optical density units at an optical density of 0.300 and at a wavelength of 530 mμ. This is well within the standard error of the method. The adapter on the Model 6A Coleman Spectrophotometer was modified by reducing the vertical light path to 15 mm. in order to accommodate a 4.5 ml. sample.

Two blanks are required. One blank consists of 5% trichloroacetic acid and standard reagent in proportions corresponding to those used for the reaction. This blank is used to establish the zero optical density setting of the spectrophotometer. The second blank consists of 1.5 ml. of serum hydrolyzate and 3.0 ml. of acetic sulfuric acid mixture[5] without diphenylamine. The second blank must be prepared separately for each serum sample. The optical density of this blank is subtracted from the corresponding serum sample giving the purple color reaction.

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[4] Composition of sensitive reagent: glacial acetic acid 90 ml., conc. sulfuric acid 10 ml., diphenylamine 1 gm.

[5] Composition: glacial acetic acid 100 ml., conc. sulfuric acid 2.75 ml.

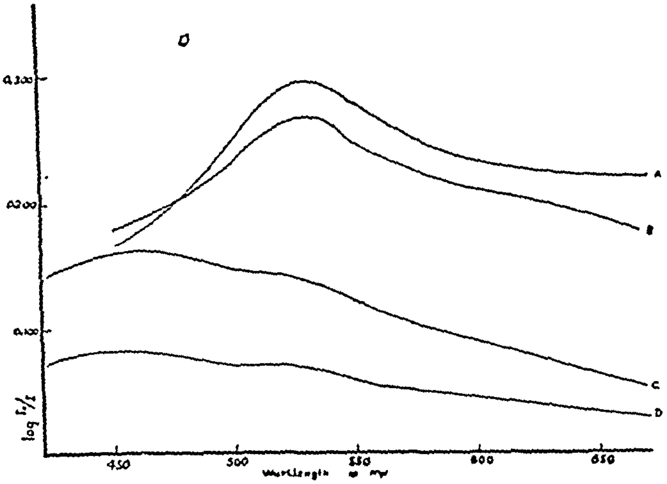

FIG. 2. COMPARISON OF THE ABSORPTION CURVES OBTAINED USING THE "SENSITIVE" AND THE "STANDARD" REAGENTS AND MIXTURES

Measurements made on a Model DU Beckman Spectrophotometer.

A. with sensitive reagent
B. with standard reagent
C. with sensitive mixture *
D. with standard mixture *

* Acetic and sulfuric acids in the proportions used in the reagents but without diphenylamine.

This furnishes the corrected optical density of the purple color determination.

A mixture of sulfuric and acetic acids in the proportions used in the reagent, when added to serum hydrolyzate, gives a light brown color with a broad absorption band as is seen in Figure 2. The sulfuric acid in the mixture causes this color development. Since the intensity of this color varies with individual serum samples it must be subtracted from the measured optical density of the purple color reaction.

*b*) *Semimicro procedure:* For small serum samples, the following procedure is suggested: To 0.2 ml. of the sample (serum or plasma) in an 18 × 150 ml. o.d. test tube add 5 ml. of a 5% trichloroacetic acid solution, slowly and with shaking. The method described in the macro procedure above is followed with one exception. In the micro technique "sensitive reagent" is used instead of standard reagent, and "sensitive mixture"[6] replaces the standard mixture.

## *Reproducibility of the results*

The arithmetic mean of the optical densities of 20 samples of the *same* serum using the macro procedure was 0.440 with a standard deviation of 0.018. The coefficient of variation was found to be 4.09%. We have expressed the results of the reaction in terms of optical density at 530 mμ since the molecular structure of the substances responsible for the purple color development in serum are as yet unknown. Evidence to be discussed elsewhere (5) suggests that the purple color development is due to a carbohydrate moiety bound mainly, if not exclusively, to the serum mucoproteins, and found in the alpha globulin fraction of serum. The arithmetic average of the optical density of serum samples from 17 normal adults (male and female) using the *macro procedure*, is 0.374, with a standard deviation of 0.041.

We have established the normal range of optical densities by using twice the standard deviation on either side of the arithmetic mean. The normal range of optical density is found to be from 0.292 to 0.456. Since our procedure differs from that used by Niazi and State (1), our values are not directly comparable. Nevertheless, the values obtained by Niazi and State differ but slightly from the values reported above.

The arithmetic average of the optical density of serum samples from 14 normal adults (male and female) using the *semimicro procedure,* is 0.332, with a standard deviation of 0.022. The normal range of values thus becomes 0.288 to 0.376 using this procedure.

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[6] Composition: glacial acetic acid 90 ml., conc. sulfuric acid 10 ml.

### *Application of the method to rheumatic fever*

The plasma or serum of rheumatic fever patients has been tested using the macro procedure previously described. We find that both serum and plasma give the same color intensity within the experimental error of the method.

Blood samples were collected from patients, either in the fasting or post-prandial state, on the wards of the Children's Memorial Hospital[7] and from ambulatory patients attending the Bridgeport Clinics.[8] When serum was used this was centrifuged from the clot as soon as possible and the reaction was carried out immediately. If this was not practicable, samples were stored at 5° C.; storage at this temperature for as long as six days does not affect the color yield. When plasma was used, heparinized blood samples were collected and centrifuged as soon as possible. Heparin gives a negative color test and does not interfere with the reaction.

We have tested a total of 107 samples from rheumatic patients in different stages of the disease. The results are recorded in Table I. The figures in Table I show that in acutely ill patients, the mean values of the reaction are significantly high (62.8% higher than the normal mean).

Figure 3 represents graphically the distribution of values found in the clinically active group of cases, around the arithmetical mean of the group. For comparison the values of normals are similarly represented.

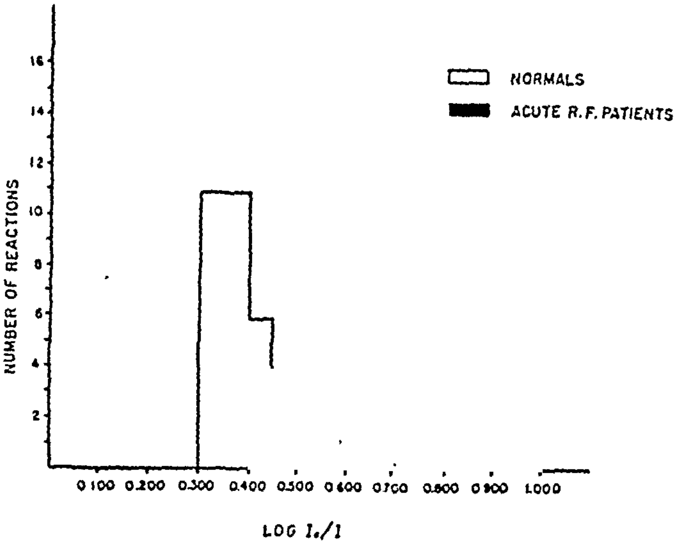

FIG. 3. DISTRIBUTION OF PURPLE COLOR VALUES OF NORMAL AND RHEUMATIC SERA, USING THE MACRO PROCEDURE DESCRIBED

TABLE I

*Purple color reaction values found in sera of rheumatic fever patients (in terms of optical density at 530 mμ)*
*(Macro procedure)*

| Number of cases | Stage | Arithmetic mean of the group | Per cent increase over normal mean |
|---|---|---|---|
| 40 | Clinically acute cases | 0.609 | 62.8 |
| 33 | Cases with only mild activity | 0.530 | 41.7 |
| 34 | Inactive cases | 0.420 | 12.3 |

The second group in Table I includes patients who had no clinical symptoms of rheumatic activity but still had slightly elevated temperatures or had sedimentation rates above normal. In this group also, the arithmetical mean of the values was significantly higher than normal levels but in a lower range than the first group.

The third group is made up of quiescent rheumatic subjects. The arithmetical mean of this group, although above the normal mean (12.3%), fell within the normal range.

The purple color reaction appears to be significantly elevated during acute rheumatic fever, to decrease with lessening of the rheumatic activity and to return to normal upon recovery. Furthermore, the intensity of the reaction appears to parallel the erythrocyte sedimentation rate except where the E.S.R. was depressed during congestive heart failure. Under these conditions the purple color reaction parallels rheumatic activity.

## SUMMARY

1. The conditions of a reaction between serum and diphenylamine reagent have been studied. Based upon these studies a simplified procedure for carrying out the reaction is recommended. With the procedure the arithmetic mean of the optical densities for normal human serum is 0.374.

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[7] We are indebted to the Children's Memorial Hospital for providing these samples.

[8] Clinics for rheumatics maintained by the Rheumatic Fever Research Institute at the Bridgeport Salvation Army Dispensary.

The standard deviation is 0.041, and the range of values for normal human serum is shown to be 0.292 to 0.456.

The composition of a more sensitive reagent is also given. With this sensitive reagent it is possible to make determinations with 0.2 ml. of serum or plasma. The arithmetic mean of the optical densities for normal human serum is 0.332 in the semimicro procedure. The standard deviation is 0.022 and the range of values for normal human serum is 0.288 to 0.376.

2. One hundred and seven blood samples from patients in different stages of rheumatic fever have been tested with the macro procedure. The purple color level in serum or plasma was found to be significantly elevated during rheumatic activity. However, in inactive rheumatic subjects the mean of the group was found to be within normal range. Serial determinations with this test may be used as an adjunct to current methods for following the course of rheumatic activity.

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# THE EFFECT OF HEXAMETHONIUM AS COMPARED TO PROCAINE OR METYCAINE LUMBAR BLOCK ON THE BLOOD FLOW TO THE FOOT OF NORMAL SUBJECTS[1,2]

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(Submitted for publication March 20, 1951; accepted May 7, 1951)

In 1948, Paton and Zaimis reported on a series of polymethylene bistrimethyl ammonium compounds with unusual properties; the decamethonium (C10) member of this series exhibited marked curariform activity, while the penta- and hexamethonium (C5 and C6) compounds revealed ganglionic blocking action (1, 2). Since this report, a number of clinical studies have called attention to the favorable effects of C5 and C6 in hypertension (3–6), peptic ulcer (7) and peripheral vascular diseases (4, 8, 9). Investigations in this laboratory indicated that in a cool environment C6 produced a greater elevation of skin temperature of the digits than either tetraethylammonium salts or Priscoline (9). In addition, C6 caused less side effects and had a longer duration of action than either of the other blocking agents.

These results suggested that the increase in peripheral blood flow after administration of C6 was greater than was produced by previously known compounds and might, in fact, approach that achieved following complete blockade of the sympathetic vasoconstrictor outflow. Since the changes in skin temperature provide at best only approximate estimations of variations in peripheral blood flow, it was decided to measure quantitatively the increase of blood flow in the foot after C6, using the plethysmographic method, and to compare this increase with the presumably maximum flow obtained from regional block of the sympathetic nerves to the lower extremities.

## MATERIAL AND METHODS

Blood flow was measured in the left foot using the venous occlusion plethysmograph described by Abramson (10), but with certain modifications. These changes were introduced because of the necessity of removing the foot from the plethysmograph at the time of the regional block and then replacing it rapidly to avoid delaying the determinations of blood flow. Therefore, instead of cementing the edge of a thick rubber sheeting to the ankle, a thin rubber boot was prepared, the edges of which were everted and sealed permanently to the inlet of the plethysmograph in a manner similar to that described by Krogh, Landis and Turner (11) for use with the limb segment plethysmograph. The boot was made of rubber thin and pliable enough for the pressure of the water filling the plethysmograph to press the rubber membrane snugly against the contours of the foot.[4] Since an air pocket usually formed at the upper end of the boot, the air was removed by means of small-bore plastic tubing leading from the toe of the boot to one of the two outlets of the plethysmograph. The trapped air could then be removed by applying suction to the plastic tube with a syringe. By means of this device, the foot could be sealed quickly within the plethysmograph, and valid blood flows recorded within 15 minutes after the block had been completed. The smoked drum method of recording was replaced by an electrically heated writing point attached to the Brodie's bellows and the recordings made on heat-sensitive paper.[5]

Digital pulse volume and blood flow were measured in the first toe of the opposite foot with a Burch-Winsor portable plethysmograph (12). Venous occluding cuffs for these measurements were placed at either the base of the toe or the ankle. The results of these studies will be reported in detail in another communication (13). In order to check on the completeness of sympathetic paralysis, the digital plethysmograph was used to determine sympathetic vasoconstrictor reflexes in the toe to "noxious" stimuli. The skin temperature of eight points on the body as well as air temperatures were recorded by the method previously described (9).

The 10 volunteer subjects, all healthy male medical students between 22 and 29, were lying comfortably in bed

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[1] Supported in part by research grants from the National Heart Institute, National Institutes of Health, U. S. Public Health Service; The Squibb Institute for Medical Research, New Brunswick, N. J.; and Irwin, Neisler and Co., Decatur, Ill.

[2] Hexamethonium was supplied by Dr. H. Sidney Newcomer, E. R. Squibb & Sons, New York City.

[3] Captain, M.C., U.S.A.F.

[4] Meteorological balloon rubber, supplied by Dewey and Almy Company, Cambridge, Mass.

[5] Permapaper, Sanborn Company, Cambridge, Mass.

with the limbs exposed throughout the procedure. They were unclothed except for shorts. The room was kept between 68 and 70 degrees by a large water-cooled air conditioner. The water in the plethysmograph was kept at a constant 32 degrees C. by means of a built-in heater and a motor-driven stirring propeller. Each subject was allowed to gain equilibrium with the room temperature for at least one hour before the first control determinations. In order to minimize the possibility of profound hypotension after the administration of C6, the foot of the bed was elevated on 2-inch blocks in all procedures.

Regional block was carried out by using lumbar epidural injection of metycaine rather than paravertebral injection of procaine because of the greater certainty of achieving complete paralysis of all sympathetic vasoconstrictor fibers to the feet. To make certain that complete paralysis had been achieved two of the subjects were later given spinal anesthesia.[6] All subjects given lumbar epidural block experienced hypesthesia of the lower extremities, and two developed anesthesia of the saddle area as well. Both volunteers subjected to intrathecal block experienced complete anesthesia to the level of the iliac crests.

In order to permit the effects of the preceding experiment to wear off completely in each subject, an interval of at least 48 hours separated the test using C6 and the procedure using regional block. The two subjects who received lumbar intrathecal block were allowed at least seven days between experiments.

Before each procedure, eight to 16 recordings of blood flow through the foot were averaged to obtain the control value. After either regional block or C6, groups of four to 10 flow measurements were recorded at various intervals up to two hours or until the skin temperature began to fall. C6 was administered intravenously as the dibromide salt. However, all dosages refer to the amount of C6 ion rather than of the salt.

## RESULTS

### *Changes in foot blood flow*

During the control period the blood flow in the foot ranged from 0.3 to 2.7 cc. per 100 cc. of foot volume per minute, with an average of 1.0 cc. (Table I, Figure 1). In most subjects the mean blood flow in the control period varied slightly from test to test but these variations never exceeded 1 cc. per 100 cc. per minute.

Following the injection of hexamethonium, the blood flow varied between 6.2 and 13.2 cc. per 100 cc. per minute, with a mean flow of 9.8 cc. (Table I, Figure 1). Following epidural block in these subjects, the flow averaged 10.2 cc. with a range of 7.1 to 15.8 cc. After C6 the maximum blood flow in five of the subjects was slightly greater than that achieved by epidural block, and in the other five slightly less.

The rate of increase of blood flow in the foot was always much more rapid after C6 than after epidural block. Maximum blood flow occurred five to 15 minutes after the intravenous administration of C6, whereas the greatest increase in blood flow after epidural block did not become evident for 25 minutes to an hour or more (Figure 2). The rate of decrease of foot blood flow following C6 varied considerably in different subjects. One hour after injection the mean flow was 74.5% of the maximum with a range of 45% to 98%. Following epidural block the rate of decrease of foot blood flow appeared to be more gradual than after C6, although, because of the delay in attaining maximum levels, the studies were not continued for a sufficient time to obtain quantitative data in all cases. Of the six cases who following epidural block attained maximum flow in less than 45 minutes, the mean flow 60 minutes after block was 76.3% of the maximum with a range of 28% to 98%.

In two subjects (J. L. and P. S., Table I), the initial experiments indicated that the increased flow of blood following 50 mg. of C6 was considerably less than that after epidural block. Because of this discrepancy, determinations of blood flow were repeated in these two subjects, using larger doses of hexamethonium. Following a dose of 75 mg. of C6 in subject J. L., the flow rose to 13.2 cc. per 100 cc. of foot volume, as compared to 8.2 cc. after the 50 mg. dose. In subject P. S. blood flow increased to 7.1 cc. after 100 mg. of C6 as compared to 5.3 cc. after 50 mg. of the drug. Thus, in both these subjects doses of C6 larger than 50 mg. were required to produce elevations of blood flow approaching the maximum.

In addition to the studies after C6 as compared to epidural block, two of the volunteers were subjected also to intrathecal anesthesia (cases L. I. and V. T., Table I and Figure 1). In subject V. T., blood flow rose to slightly higher levels after intrathecal anesthesia (9.8 cc. per 100 cc. of foot) than after either C6 (8.3 cc.) or epidural block (7.5 cc.). In subject L. I., however, the reverse occurred; the maximum blood flow was only 6.2 cc. after intrathecal anesthesia as compared to 8.7 cc. after epidural block and 10.0 cc. after C6.

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[6] Metycaine 1% was used for all epidural lumbar blocks. Procaine 1% was used for all intrathecal blocks.

TABLE I

*Effects of hexamethonium bromide, lumbar epidural and intrathecal anesthesia on foot blood flow in normal subjects*

| Subject | Age | Foot blood flow (cc. per 100 cc. of foot per minute) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Control | After C6 50 mg. I.V. | Control | After epidural | Control | After intrathecal |
| V. T. | 25 | 1.2 | 8.3 | 0.9 | 7.6 | 0.8 | 9.8 |
| L. I. | 24 | 0.4 | 10.0 | 0.8 | 8.7 | 1.0 | 6.2 |
| L. D. | 28 | 0.3 | 11.6 | 0.6 | 9.1 | | |
| V. B. | 25 | 0.9 | 9.2 | 0.6 | 8.8 | | |
| J. L. | 22 | 1.2 | 8.2 | 0.7 | 15.8 | | |
| | | 1.0 | 13.2* | | | | |
| W. B. | 27 | 1.1 | 6.2 | 1.7 | 7.1 | | |
| S. O. | 27 | 0.8 | 10.8 | 1.5 | 12.6 | | |
| J. D. | 28 | 2.7 | 9.2 | 1.7 | 11.8 | | |
| R. B. | 29 | 1.0 | 12.8 | 1.0 | 9.4 | | |
| P. S. | 23 | 0.7 | 5.3 | 0.5 | 11.2 | | |
| | | 0.7 | 7.1† | | | | |

* Dosage of C6 increased to 75 mg. intravenously.
† Dosage of C6 increased to 100 mg. intravenously.

### *Changes in skin temperature of the toes*

In every instance after either C6 or regional block with procaine, there was a marked rise of the skin temperature in the toes (Figure 3). These elevations varied between 15 and 24 degrees F. above the control values. The elevations of toe temperature were greater after epidural block than after hexamethonium in seven of the nine subjects in whom measurements were taken. In all but one (L. D.), however, the differences in skin temperature following C6 as compared to regional block were slight, amounting to less than 2 degrees F.

The changes in digital skin temperature correlated poorly with the changes in blood flow. In subjects P. S. and W. B., for example, the rise in temperature was greater after C6, yet the increase in blood flow was greater after epidural block. Similar contradictory results were obtained in three other subjects.

The rate of elevation of skin temperature proved also an unreliable index of the rapidity of increase in blood flow. Following regional block, the increase in skin temperature of the toe and foot lagged five minutes or more behind the rise in

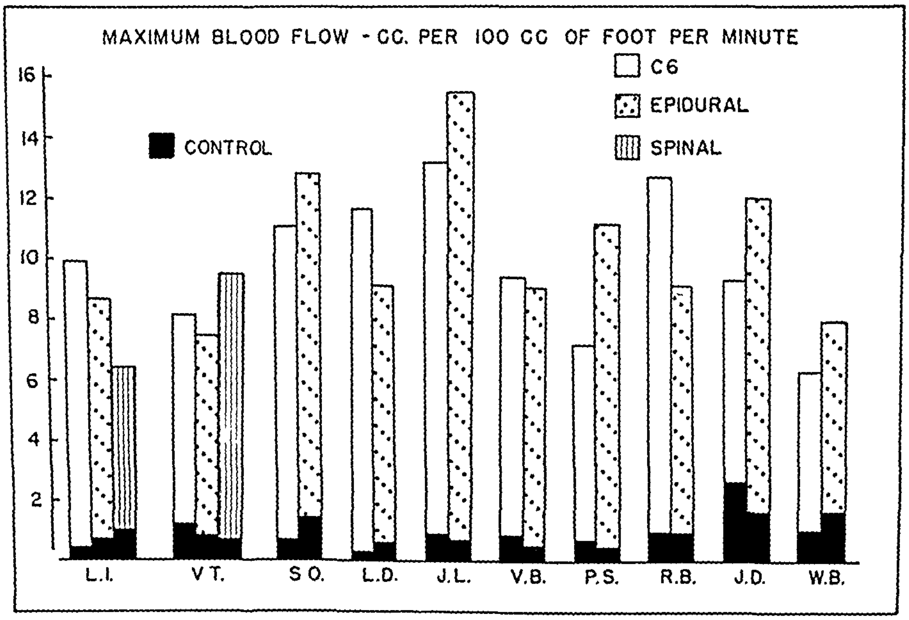

FIG. 1. CHART OF THE MAXIMUM BLOOD FLOWS IN THE LEFT FOOT FOLLOWING HEXAMETHONIUM AS COMPARED TO LUMBAR INTRATHECAL OR EPIDURAL BLOCK IN 10 NORMAL SUBJECTS

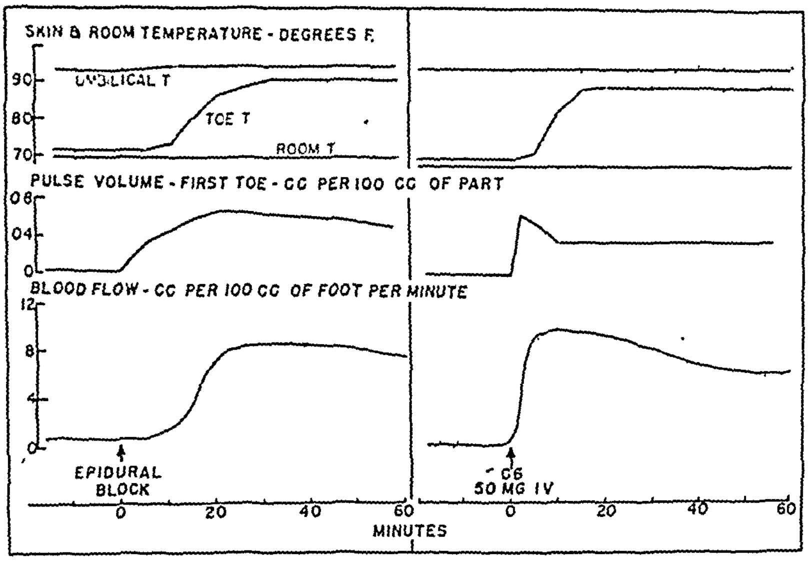

FIG. 2. CHART SHOWING CHANGES IN SKIN TEMPERATURE, DIGITAL PULSE VOLUME AND FOOT BLOOD FLOW IN SUBJECT L. I. FOLLOWING EPIDURAL BLOCK AND FOLLOWING C6

blood flow. Furthermore, the skin temperature remained elevated for an additional 15 or 20 minutes after the plethysmographic tracings showed that the foot blood flow had passed the maximum and was decreasing.

### *Changes in vasoconstrictor reflexes in the toes*

The changes in blood flow in the toe measured with the digital plethysmograph paralleled those in the foot. However, the digital pulse volume always exhibited a greater increase after regional block than after C6. In all instances, the sympathetic vasoconstrictor responses to "noxious" stimuli (such as a deep breath or ice applied to the forehead) were effectively blocked during the period of maximum elevations of foot blood flow following either epidural or intrathecal anesthesia or C6.

## DISCUSSION

Investigations by Hoobler and his co-workers using the water-filled plethsymograph indicated that in normal subjects large doses of tetraethylammonium produced increases in foot blood flow only half as great as those obtained after lumbar paravertebral block with procaine (14). Applying heat to the trunk produced no greater increase in blood flow in the foot than did the administration of TEA, while other vasodilator drugs such as papaverine, nitroglycerine, nicotinic acid, and aminophyllin were even less effective.

In the present study the increases in foot blood flow following 50 to 100 mg. of C6 intravenously were not greatly different from those after either epidural or intrathecal block using metycaine or procaine. These results suggest that the degree of blockade of the sympathetic vasoconstrictor impulses to the feet is nearly complete after the intravenous injection of such doses of C6.

There is some evidence, however, that regional block produced a slightly greater decrease in peripheral resistance than C6. The mean of the foot blood flow values was 10.2 cc. per 100 cc. of foot volume after regional block, which was slightly greater than the mean of 9.8 cc. after C6. This difference, however, was not statistically significant. Similarly, the skin temperature elevations in the toes usually were slightly higher following regional block. In addition, it was observed that the pulse volume of the toe was consistently greater after regional anesthesia than after C6, even in those cases in which C6 produced the greater increase in foot and toe blood flows. This discrepancy, which will be discussed in detail in another communication (13), appeared to be associated with a decrease of systolic blood pressure and increase of heart rate resulting in narrowing of pulse pressure observed after C6 but not after lumbar block. In view of these various observations, it seems possible that the degree of sympathetic in-

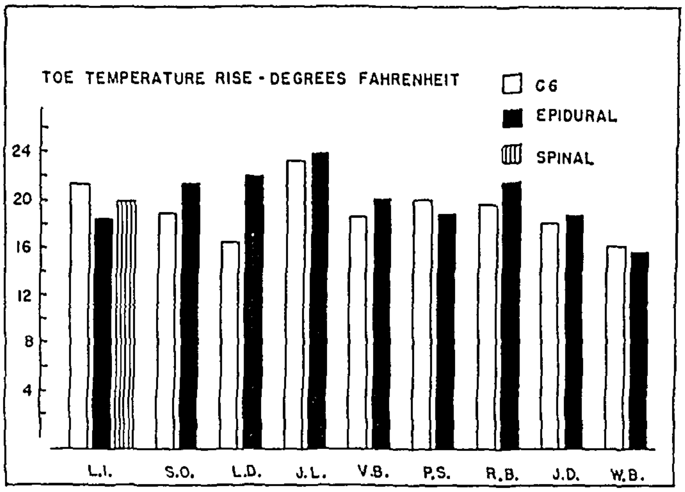

FIG. 3. CHART SHOWING THE MAXIMUM RISE OF TOE TEMPERATURE FOLLOWING C6 AS COMPARED TO EPIDURAL OR INTRATHECAL ANESTHESIA IN 10 NORMAL SUBJECTS

hibition after C6 approached but may not have achieved complete blockade in some of the subjects.

Nevertheless, these observations would appear to be at odds with the hypothesis of hemometakinesia, that is, the so-called "borrowing and lending" theory. This hypothesis states in part that the systemic administration of any vasodilating agent cannot result in as great an increase in foot blood flow as does regional block (15, 16). After regional anesthesia, blood from other portions of the body is shunted into the area of diminished resistance. However, according to this hypothesis, after systemic administration of a vasodilating agent blood cannot be diverted to any local area because the decrease in peripheral resistance is generalized.

While this hypothesis may apply to agents which act directly on the blood vessels, it is probable that the "sympatholytic" compounds act more selectively. Sympathetic vasoconstrictor nerves probably are not distributed uniformly throughout the vascular system. Actual measurement of regional blood flows after other drugs which inhibit sympathetic vasoconstrictor impulses, such as TEA (17, 18) and dihydroergocornine (19), indicates that whereas blood flow in the digits usually increases markedly, flow in the muscles and renal bed may not change significantly and hepatic-portal blood flow usually decreases. Cardiac output remains relatively unchanged (17, 19). Thus peripheral resistance in certain vascular areas such as the digits may decrease after the "sympatholytic" drugs while in other areas such as the hepatic-portal bed it may increase. Hence, as indicated by the present studies, a considerable degree of "borrowing and lending" of blood flow may occur from the action of agents which are sufficiently potent to produce complete or nearly complete blockade of sympathetic vasoconstrictor impulses.

It is apparent from the data derived from subjects J. L. and P. S. that doses of 75 to 100 mg. of C6 in some persons produce more complete ganglionic blockade than 50 mg. Hence, it would seem advisable to give 100 mg. in various clinical states when complete inhibition of sympathetic vasoconstriction is desired. In severely hypertensive, cardiac, and debilitated patients, however, profound and probably dangerous hypotension may occur after such large doses. Therefore, in actual practice, trial doses of 5 to 10 mg. have been given to these patients before increasing the dose to maximum tolerated amounts (20).

None of the subjects of the present study exhibited significant reduction of diastolic blood pressure as a result of either epidural block or hexamethonium, although there usually was a slight decrease in systolic pressure. The absence of significant hypotension in these normal, healthy young men could not be due entirely to the slightly head-down position because we have observed marked reductions of arterial pressures in hypertensive and older normotensive patients after similar doses of C6 despite this precaution (9). These results may be due to (*1*) the presence of a higher sympathetic "tone" in older as compared to younger individuals, or (*2*) to the loss of elasticity of the larger vessels in the elderly resulting in a lessened ability to counteract sudden decreases in peripheral resistance.

Because of its great potency C6 is capable of producing severe reductions of blood pressure in susceptible patients. In addition, marked postural hypotension has been a regular occurrence. During continuous administration effects secondary to parasympathetic blockade have been observed, including constipation, paralytic ileus and dry mouth. These undesirable reactions require that the drug be administered with care in patients under treatment for peripheral vascular diseases and hypertension. Methods for avoiding or circumventing such reactions in clinical cases will be described in other communications (20, 21).

## SUMMARY AND CONCLUSIONS

Ten normal young male subjects in a constant, cool temperature room were subjected on alternate days either to 50 to 100 mg. of C6 ion intravenously or to lumbar extradural or intrathecal block.

1. Five of the subjects exhibited a greater increase in foot blood flow after C6 while five others had the greater flow after regional block.
2. Evidence that the sympathetic blockade produced by C6 may not have been quite complete is suggested by the fact that in the group as a whole the averages of the foot blood flow, toe blood flow and digital skin temperature changes were slightly greater after regional block than after C6. In some of the subjects, however, the values rose higher after C6 than after regional block.
3. Changes in skin temperature and digital pulse volume did not accurately reflect the changes in foot blood flow.
4. In two subjects doses of 75 and 100 mg. of C6 resulted in greater elevations of foot blood flow than doses of 50 mg.

These results suggest that C6 in doses of 50 to 100 mg. produces in man marked, and in some instances, complete blockade of the sympathetic vasoconstrictor outflow to the foot.

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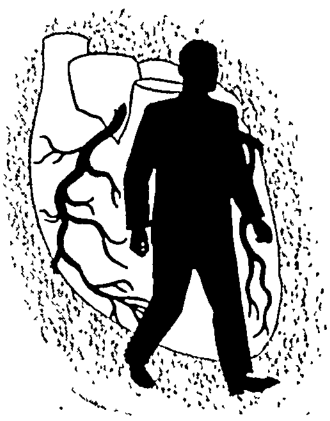

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# THE EFFECTS OF TWENTY DEGREE HEAD-UP TILT UPON THE CEREBRAL CIRCULATION OF PATIENTS WITH ARTERIAL HYPERTENSION BEFORE AND AFTER SYMPATHECTOMY[1]

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(Submitted for publication October 5, 1950; accepted May 23, 1951)

The present study was undertaken in order to measure the effect of a slight elevation of the head and shoulders upon the cerebral circulation of hypertensive patients following sympathectomy. Twenty degree tilt was chosen because it could be tolerated for 20 minutes, as early as two weeks after the Smithwick sympathectomy.

Shenkin and coworkers in 1948 reported that 20° head-up tilt did not alter the cerebral blood flow, cerebral oxygen consumption, and oxygen content of the internal jugular blood in five normal subjects (1). They also reported that, following thoracolumbar sympathectomy, cerebral oxygen uptake and blood flow in hypertensive patients while in the supine position did not differ from the preoperative level (2).

We have measured mean arterial pressure, blood flow, and oxygen uptake of the brain, before and after tilt in two groups of patients: (*a*) 15 patients who had been subjected to bilateral thoracolumbar (D6–L3) sympathectomy, and (*b*) 18 patients with essential hypertension.

## METHODS

Patients with essential hypertension were selected from the medical and surgical wards of the Hospital of the University of Pennsylvania and the Graduate Hospital of the University of Pennsylvania. Preoperatively all had moderate to marked hypertension with retinopathy[5] grades II to IV (3), fluoroscopic and electrocardiographic evidences of left ventricular hypertrophy, and moderate impairment of renal function as measured by PSP excretion and urea clearance tests. At the time of the tilt study no patient showed either elevation of the blood urea nitrogen above 20 mgm. % or signs of congestive heart failure.

The patients studied after sympathectomy were selected at random without regard to the severity of the postoperative postural hypotension. All were ambulatory. Sympathectomies had been completed from two weeks to seven years prior to testing. Ten of the 17 tilts following sympathectomy were made in the 12 months immediately after operation, and of these ten, eight were studied within six months after operation.

Control measurements of cerebral blood flow, oxygen consumption, and mean femoral arterial pressure (4) were made in the supine position in a room kept as cool as was consistent with the patient's comfort. Accurate control of the environmental temperature was not possible. The patient was then tilted to a 20° head-up position. After three minutes in this position, simultaneously drawn samples of blood from the femoral artery and the internal jugular vein were obtained for analyses of oxygen and carbon dioxide content and blood pH as previously reported (5). When the patient had been in the head-up position for 20 minutes the second cerebral blood flow and oxygen consumption measurement was made. The pressure in the femoral artery during tilt and the vertical distance from the position of the needle to the level of the patients' eyes were recorded. The cerebral arterial pressure was then calculated as reported by Scheinberg and Stead (6). The jugular venous pressure was then subtracted from the calculated cerebral arterial pressure to give "effective" cerebral arterial pressure. Ve-

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[1] This study was supported in part by research grants from the National Heart Institute, U. S. Public Health Service, the Life Insurance Medical Research Fund, Sandoz Pharmaceuticals, Irwin, Neisler and Company, and the Squibb Institute for Medical Research.

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[5] These examinations were made by Dr. Irving H. Leopold, Department of Ophthalmology, Hospital of the University of Pennsylvania.

nous pressure was measured (at the level of the internal jugular bulb) both while in the supine position and at the end of the tilt period while still semi-erect.

RESULTS

Table I contains the data on each patient. Also included are values previously reported for the normotensives. The percentage change and the significant differences from the supine value in each group are given in Table II. There were no highly significant differences between the mean differences of the three groups in any of the variables.

### *1. Mean arterial pressure*

The "effective" cerebral mean arterial pressure was decreased by tilt in both groups of patients.

Eight patients tilted within six months or less after sympathectomy showed a highly significant change only in "effective" cerebral arterial pres-

TABLE I

*Effects of 20° head-up tilt on cerebral circulation and oxygen metabolism*

| Patient group | Retinopathy grade | "Effective" cerebral mean arterial pressure, *mm. Hg** | | | Oxygen content internal jugular vein, *vol.%* | | | Arteriovenous oxygen difference, *vol.%* | | | Cerebral oxygen uptake, *cc./100 G./min.* | | Cerebral blood flow, *cc./100 G./min.* | | Vascular resistance† | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | S | 3′ | 20′ | S | 3′ | 20′ | S | 3′ | 20′ | S | 20′ | S | 20′ | S | 20′ |
| *Essential hypertension* | | | | | | | | | | | | | | | | |
| H. H. 48 w m | III | 144 | 118 | 116 | 9.9 | 10.5 | 10.0 | 4.5 | 4.3 | 4.4 | 3.5 | 3.2 | 77 | 73 | 1.9 | 1.6 |
| D. L. 19 w f ‡ | IV | 141 | 128 | 112 | 12.0 | 11.5 | 11.0 | 4.7 | 5.4 | 5.6 | 3.3 | 4.0 | 70 | 71 | 2.0 | 1.6 |
| W. R. 35 c m | II | 114 (4.9)§ | 101 | 96 (4.1)§ | 11.1 | 10.7 | 9.8 | 5.4 | 6.0 | 6.9 | 3.1 | 3.6 | 58 | 52 | 2.0 | 1.8 |
| W. M. 40 w m‡ | IV | 144 (9.2) | 128 | 121 | 12.1 | | 12.7 | 5.0 | | 5.9 | 3.1 | 3.7 | 62 | 63 | 2.3 | 1.9 |
| A. R. 33 w f ‡ | II | 130 | | 126 | 9.7 | | 9.2 | 8.8 | | 9.9 | 4.0 | 4.1 | 45 | 41 | 2.9 | 3.1 |
| A. S. 50 w m | III | 127 | | 113 | 10.5 | | 8.9 | 6.3 | | 8.3 | 3.1 | 3.7 | 49 | 45 | 2.6 | 2.5 |
| N. M. 49 c f | III | 147 (8.1) | 113 | 104 (3.7) | 10.8 | 10.5 | 9.5 | 5.9 | 5.6 | 6.2 | 2.9 | 3.7 | 49 | 60 | 2.8 | 1.7 |
| H. M. 45 w f | IV | 192 (2.9) | 180 | 162 (2.2) | 10.0 | 10.4 | 9.3 | 7.2 | 6.7 | 7.5 | 3.3 | 3.3 | 47 | 44 | 4.1 | 3.7 |
| J. P. 43 w m | IV | 151 (5.6) | 114 | 128 (5.9) | 9.6 | 8.3 | 8.5 | 5.2 | 6.4 | 6.4 | 2.3 | 2.9 | 48 | 45 | 3.1 | 2.8 |
| E. K. 29 w f | II | 129 (6.5) | 110 | 111 (5.0) | 12.7 | 12.3 | 12.2 | 5.4 | 5.6 | 5.6 | 5.0 | 5.1 | 93 | 91 | 1.4 | 1.2 |
| W. W. 45 c m | II | 151 (8.2) | 130 | 138 (8.7) | 11.9 | 11.8 | 10.9 | 5.7 | 6.8 | 6.4 | 4.0 | 4.2 | 70 | 65 | 2.2 | 2.1 |
| L. B. 31 c f ‖ | III | 149 (6.2) | 143 | 134 | 8.1 | 7.4 | 7.5 | 4.9 | 5.3 | 5.3 | 4.5 | 3.6 | 91 | 68 | 1.6 | 2.0 |
| V. K. 41 w f | II | 151 (8.1) | 139 | 134 | 10.4 | 10.6 | 10.5 | 7.3 | 6.5 | 7.1 | 4.7 | 4.7 | 64 | 66 | 2.4 | 2.0 |
| R. A. 45 c f ‖ | II | 149 | | 98 | 12.4 | | 11.3 | 6.0 | | 6.3 | 4.1 | 4.5 | 68 | 72 | 2.2 | 1.4 |
| S. B. 43 w f | II | 159 | 124 | 123 | 11.3 | 10.1 | 10.2 | 6.2 | 6.8 | 6.7 | 3.6 | 3.8 | 58 | 56 | 2.7 | 2.2 |
| W. B. 52 c m‖ | III | 156 | 166 | 111 | 8.4 | 9.9 | 7.8 | 4.9 | 5.3 | 5.5 | 3.3 | 3.4 | 67 | 61 | 2.3 | 1.8 |
| A. T. 44 c f | III | 151 (3.7) | 132 | 130 (2.9) | 10.4 | 8.7 | 9.2 | 6.3 | 7.5 | 7.6 | 2.6 | 3.3 | 42 | 43 | 3.6 | 3.0 |
| J. D. 41 w m | II | 147 | 140 | 137 | 11.8 | 11.4 | 11.4 | 8.2 | 7.6 | 7.9 | 2.7 | 2.8 | 33 | 36 | 4.5 | 3.8 |
| Mean | | 146 (5.7) | 131 | 122 (4.6) | 10.7 | 10.2 | 10.0 | 6.0 | 6.1 | 6.6 | 3.5 | 3.8 | 61 | 58 | 2.6 | 2.2 |
| Standard dev. indiv. diff. | | | | 13 (1.7) | | 0.7 | 0.6 | | 0.8 | 0.6 | | 0.4 | | 7 | | 0.3 |
| *Postsympathectomy* (*mos.*) | | | | | | | | | | | | | | | | |
| Y. J. 46 w f 6½ | I | 123 | | 92 | 10.9 | 9.9 | 9.6 | 4.7 | 6.5 | 6.5 | 3.4 | 4.0 | 72 | 62 | 1.7 | 1.5 |
| M. H. 33 w f 17 | II | 135 (9.1) | 104 | 49 (6.0) | 10.4 | 9.8 | 6.6 | 6.9 | 6.5 | 11.2 | 5.3 | 5.0 | 77 | 44 | 1.8 | 1.1 |
| F. H. 33 w m 16 | II | 114 | 92 | 91 | 12.1 | 11.9 | 11.4 | 5.3 | 6.2 | 6.5 | 3.3 | 3.3 | 62 | 51 | 1.8 | 1.8 |
| P. B. 46 w m 7 | II | 104 (5.9) | 98 | 98 | 10.7 | 10.7 | 10.1 | 6.3 | 6.8 | 7.0 | 3.3 | 4.1 | 52 | 59 | 2.0 | 1.7 |
| A. P. 32 w f 21 | I | 110 (9.1) | 115 | 102 | 8.3 | 9.0 | 7.9 | 8.0 | 6.9 | 8.0 | 3.7 | 3.6 | 46 | 45 | 2.4 | 2.3 |
| E. L. 43 w m 6 | II | 159 (8.1) | 141 | 127 (7.4) | 10.4 | 11.1 | 10.6 | 6.3 | 8.5 | 6.3 | 2.5 | 2.8 | 40 | 45 | 4.0 | 2.8 |
| S. C. 33 c f 3 ¶ | III | 168 (7.5) | 151 | 133 | 8.5 | 8.9 | 8.4 | 8.6 | 7.5. | 8.3 | 3.4 | 3.8 | 39 | 46 | 4.3 | 2.9 |
| D. L. 19 w f 3 ¶ | II | 125 (9.9) | 79 | 63 (8.8) | 10.9 | 9.7 | 9.8 | 4.5 | 5.9 | 5.9 | 2.3 | 4.1 | 50 | 69 | 2.5 | 0.9 |
| K. G. 40 w f 6 | II | 133 (4.9) | 120 | 110 (4.6) | 10.4 | 9.5 | 9.6 | 5.1 | 5.5 | 6.1 | 4.2 | 4.3 | 82 | 71 | 1.6 | 1.5 |
| W. M. 40 w m 3½¶ | II | 92 (7.8) | 83 | 83 (7.4) | 12.4 | 11.2 | 11.2 | 5.2 | 6.4 | 7.0 | 3.8 | 3.9 | 73 | 55 | 1.3 | 1.5 |
| F. K. 48 w f 3 ¶ | IV | 101 (7.1) | 100 | 60 (6.8) | 9.1 | 9.6 | 8.6 | 6.4 | 5.5 | 6.9 | 4.3 | 4.8 | 67 | 69 | 1.5 | 0.9 |
| M. D. 36 c f 7½ | II | 162 (8.5) | 135 | 101 (7.7) | 12.3 | 10.9 | 10.5 | 6.6 | 7.2 | 8.1 | 3.4 | 3.6 | 51 | 44 | 3.2 | 2.3 |
| E. W. 49 w f 60 | II | 146 (5.5) | 127 | 116 (3.3) | 10.5 | 8.2 | 7.6 | 7.1 | 8.8 | 10.1 | 2.7 | 3.2 | 38 | 32 | 3.8 | 3.6 |
| J. M. 28 w m 84 | IV | 163 (7.7) | 153 | 150 (7.5) | 10.7 | 10.2 | 11.9 | 6.7 | 8.6 | 7.3 | 5.4 | 4.2 | 81 | 57 | 2.0 | 2.6 |
| A. R. 33 w f ½ | II | 108 | | 77 | 8.4 | | 8.7 | 6.9 | | 6.6 | 3.5 | 3.0 | 51 | 45 | 2.1 | 1.7 |
| 14 | 0 | 94 | 77 | 70 | 8.7 | 7.4 | 7.8 | 6.1 | 8.0 | 7.6 | 3.9 | 3.9 | 64 | 52 | 1.5 | 1.3 |
| 24 | 0 | 95 | 77 | 59 | 9.2 | 7.6 | 7.7 | 6.0 | 7.2 | 7.3 | 3.2 | 3.9 | 53 | 53 | 1.8 | 1.1 |
| Mean | | 125 (7.6) | 110 | 93 (6.6) | 10.2 | 9.7 | 9.3 | 6.3 | 7.0 | 7.5 | 3.6 | 3.9 | 59 | 53 | 2.3 | 1.9 |
| Standard dev. indiv. diff. | | | | 21 (1.1) | | 0.9 | 1.2 | | 1.0 | 1.2 | | 0.6 | | 13 | | 0.6 |
| *Normotensive patients previously reported* (1) Mean | | 88 | | 73 | 10.3 | | 10.4 | 6.1 | | 6.3 | 3.2 | 3.3 | 53 | 54 | 1.7 | 1.4 |
| Standard dev. indiv. diff. | | | | .7 | | | 0.9 | | | 0.6 | | 0.3 | | 5 | | 0.2 |

* S signifies supine position mean values.
3′ signifies mean values after patients had been three minutes in the 20° head-up position.
20′ signifies mean values after patients had been 20 minutes in the 20° head-up position.
† Signifies "effective" cerebral mean arterial pressure divided by cerebral blood flow.
‡ Signifies patients studied after sympathectomy.
§ Signifies jugular venous pressure that was subtracted from calculated cerebral pressure to give "effective" pressure.
‖ Signifies patient had symptoms after 12 minutes head-up requiring return to supine position.
¶ Signifies patient had entire right adrenal removed at time of second stage sympathectomy.

TABLE II

*Comparison of the per cent changes in cerebral circulation and oxygen metabolism with 20° tilt*

| Patient group | Cerebral | | | | | |
|---|---|---|---|---|---|---|
| | "Effective" mean arterial pressure | Jugular oxygen content | Blood flow | Vascular resistance | Oxygen uptake | A-V $O_2$ difference |
| Essential hypertension, 18 pts., 18 observ'ns | −16* | −6* | −5 | −15* | +9* | +10* |
| Postsympathectomy, 15 pts., 17 observ'ns | −26* | −9* | −11 | −17* | +8 | +19* |
| Normotensive, 5 pts., 5 observ'ns. Prev. reported (1) | −17* | +1 | +2 | −18 | +3 | +3 |

* Signifies statistically significant differences from the mean values in the supine position ($p<0.01$).

sure. The 26% reduction was identical with that of the entire postsympathectomy group.

### 2. *Cerebral venous blood oxygen content and brain blood flow*

In both groups of patients the internal jugular blood oxygen concentration was reduced significantly from the recumbent value ($p < 0.01$). Comparable increases in the cerebral arteriovenous oxygen differences were observed. The increase in this oxygen difference after three minutes of tilt was significant in the patients studied after sympathectomy ($p < 0.02$), but was not in the patients with essential hypertension. The decreases in the oxygen content of cerebral venous blood after 20 minutes of tilt cannot be attributed to significant reductions in cerebral blood flow in either group (Table II).

### 3. *Cerebral oxygen consumption*

Although the cerebral arteriovenous oxygen difference increased only 10% in the hypertensives, the 9% increase in cerebral oxygen consumption after 20 minutes of tilt proved to be highly significant ($p < 0.01$). Cerebral oxygen consumption was not increased in the postsympathectomy group.

### 4. *Cerebrovascular resistance*

The cerebral blood flow of the patients after sympathectomy was decreased 11% with a 26% reduction in "effective" mean cerebral pressure. This can be explained by the fact that the cerebrovascular resistance decreased 17% from 2.3 to 1.9 units, indicating a highly significant relaxation of cerebral vessels during the tilt period. A comparable reduction in cerebrovascular resistance was found both in the hypertensive patients and in the normotensive subjects (1).

### 5. *Untoward symptoms during tilt*

It is noteworthy that no symptoms suggesting an inadequate cerebral circulation were encountered while the surgically treated patients were tilted. However, three patients with essential hypertension, L. B., R. A., and W. B. (Table I), having been immobile at least 12 minutes in the tilt position, suddenly developed sweating, nausea, weakness, bradycardia, marked drop in blood pressure and syncope. These were ameliorated by a return to the horizontal and the 20 minute observations in these patients were made there. R. A. and W. B. had reductions in pressure considerably greater than the mean of the group, indicating that the pressure had not returned to the pretilt level over a 15 minute period following the fainting episode. The other patient, L. B., had a 25% fall in blood flow (91 to 68) even though the pressure rose toward the control observation. There was an increase in resistance in this patient, without an appreciable increase in the cerebral arteriovenous oxygen difference. Each of these patients showed a decrease of 0.6 vol. % or more in the oxygen content of the internal jugular blood when compared with the pretilt concentration. It is of interest that L. B. had a transient left hemiplegia one month before. Some slight weakness of the left leg was present at the time of this study. The supine cerebral blood flow, cerebral arteriovenous oxygen difference, cerebral oxygen uptake, and cerebrovascular resistance were not characteristic of essential hypertension (5).

### 6. *Tilt studies before and at intervals after sympathectomy in the same patient*

Postural hypotension of a moderate degree was observed with the tilt test over the two year period following sympathectomy in patient A. R. (Table I). Although this patient had only moderate hypertension preoperatively, she was troubled with severe headaches and had evidence of vascular damage of such a degree as to warrant sympathectomy. Following operation, she had complete relief from headache. The fundi are now normal. The heart size and electrocardiogram are now normal. Renal dysfunction has not progressed.

Tilt tests were made before operation, two weeks, 14 months, and two years after sympathectomy. The cerebral blood flow during the most recent tilt study was unchanged when the "effective" mean cerebral pressure was reduced 38%. These measurements indicate that the cerebral vessels in this patient are capable of considerable relaxation at this time, cerebrovascular resistance having decreased equally with the arterial pressure. There was, however, a 25% increase in cerebral arteriovenous oxygen difference and oxygen consumption as jugular blood oxygen content decreased 1.5 vol. % when compared with the supine observation. No unpleasant symptoms were observed during this tilt period. Two other patients were studied before as well as after sympathectomy (D. L. and W. M., Table I). Cerebral blood flow increased with tilt in one, but decreased in the other. Both of these patients have had a good clinical response for at least one year after operation.

## COMMENT

These studies offer additional evidence that the high tone of cerebral vessels normally present in essential hypertension is capable of partial relaxation in response to a fall in cerebral arterial pressure induced by 20° tilt for 20 minutes. A comparable degree of relaxation, tending to counteract the decrease in cerebral blood flow which would otherwise occur, has been found in the group tilted after sympathectomy. The decrease in the resistance in the latter group during tilt appeared to augment the previously mentioned reduction in cerebrovascular resistance in the supine position after sympathectomy (2). Although the cerebral oxygen uptake of the sympathectomy group was unchanged by the 20° head-up position, there was a highly significant reduction in the oxygen content of the jugular blood in *both* groups. Kety and associates have observed a similar trend in a study of the cerebral circulatory response to the acute reduction in cerebral mean arterial pressure induced by differential spinal anesthesia (7). We interpret this as an indication that the increased tone of the cerebral vessels usually present in essential hypertension and persisting after sympathectomy could only be partly relaxed in response to the fall in pressure induced by this slight degree of tilt.

The cerebral arteriovenous oxygen difference was not increased by tilt as much in the patients with essential hypertension as in the patients tested after sympathectomy (Table II). Cerebral blood flow was reduced about the same amount. However, the 9% increase in cerebral oxygen uptake was highly significant ($p < 0.01$). This was not observed in the normotensive group. The measurements of this study offer us no explanation.

The symptoms experienced by hypertensive patients during tilt were similar to those observed during a sudden fall in arterial pressure from differential spinal sympathetic block (7). These data are further evidence suggesting that such symptoms were the result of cerebral anoxia associated with an inadequate circulation while in this position. A decrease in cerebral blood flow and an increase in the cerebral arteriovenous oxygen difference have been reported when normotensive subjects were tilted to a 65° head-up position and held there for ten minutes (6).

Our data give us no ready explanation for the difference in the symptomatic response of the two hypertensive groups to this slight degree of head-up tilt. It is probable that the absence of collapse symptoms in the postsympathectomy group was fortuitous.

The patient E. L. (Table I) was studied six months after sympathectomy. He had suffered a right hemiplegia one week after the second stage was completed. Although this patient had complete motor and sensory recovery within the next week, some weakness of the right leg remained at the time of this tilt study. E. L. had been studied before operation and there appeared to be no essential difference in the supine data, published as part of a report of the effect of stellate block (8). However, the 16% increase in blood flow during tilt with no decrease in cerebral arteriovenous difference raised the question of shunts through damaged cerebral tissue. Also of interest is the fact that the patient suffered a right homonymous hemianopia six weeks after the tilt study. This gradually cleared within three months.

Kety (9) and Schmidt (10) (Table II) have recently summarized the evidence from investigations over the past four years bearing on the relationship between arterial hypertension and the marked increase in cerebral vascular resistance accompanying it. They suggest a humoral agent as the mediator of the increased cerebral vascular resistance as well as the uniform increase in vascular tone throughout the body. The results of the present study in which hypotension was induced in the cerebral arterial system under slight physiologic stress also show that the increased cerebral vascular resistance can be decreased when the "effective" arterial pressure is lowered. The failure of the cerebral vasculature to relax to the degree enabling the oxygen content of the internal jugular vein blood to remain unchanged might be considered as further evidence suggesting the presence of a circulating pressor substance.

That relaxation of the tone of cerebral blood vessels is possible in hypertension has been demonstrated by studies of the cerebral circulation after sympathectomy (2) and following intramuscular dihydroergocornine (11). Following the administration of this drug cerebral blood flow, cerebral arteriovenous oxygen difference, and oxygen content of the internal jugular vein were essentially unchanged when the arterial pressure was significantly reduced. These latter observations seem to answer in the affirmative the question: Can the elevated arterial pressure of patients with essential hypertension be lowered without untoward symptoms that might be related to a decrease in cerebral blood flow, cerebral oxygen uptake, and mean cerebral oxygen tension?[6]

## SUMMARY

Measurements of cerebral blood flow, cerebral oxygen uptake and cerebral vascular resistance were made by the nitrous oxide method before and after 20° head-up tilt in patients with essential hypertension. A significant reduction in "effective" cerebral arterial pressure during the tilt was observed.

Cerebral blood flow was not reduced significantly during tilt in either group. Cerebral vascular resistance was reduced in both. Oxygen uptake of the brain increased when the patients with essential hypertension were tilted and was unchanged in the sympathectomy group. Symptoms of cerebral anoxia were not observed in these patients.

The oxygen content of the blood from the internal jugular vein in the two groups of patients was significantly reduced while semi-erect. This indicates that, although the cerebral vascular resistance was diminished, the increased tone was only partially relaxed.

From the data obtained we believe the following conclusions may be drawn:

1. The increased cerebral vascular resistance in essential hypertension can be partially relaxed when the arterial pressure is lowered by this slight degree of tilt.
2. Patients studied after sympathectomy showed the same degree of incomplete relaxation with tilt as the patients with essential hypertension, but did not experience cerebral anoxia.
3. Failure of the cerebral vasculature to relax completely might be considered as evidence suggesting the presence of significant amounts of circulating pressor substances even in the patients who have had a sympathectomy.

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[6] The authors recognize that venous oxygen content is a less adequate measure of cerebral oxygen homeostasis than venous oxygen saturation or tension (7).